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2.1. Stress:

“Every stress leaves an indelible scar, and the organism pays for its survival after a stressful situation by becoming a little older.”

~ Hans Selye

Stress is a complicated physiological mechanism that embodies a range of integrative physiological and behavioural processes that occur when there is a real or perceived threat to homeostasis. While it is generally accepted that these processes are adaptive, designed to re-establish homeostasis and allow coping, it is also apparent that inadequate or excessive and/or prolonged activation of stress systems can disturb normal physiological and behavioural function. This can result in a range of adverse consequences such as depression, impaired cognition, cardiovascular diseases, impaired immune function with increased vulnerability to disease, impaired growth and reproductive function, osteoporosis, diabetes, dementia and reduced life expectancy [53-58]

Despite a vast literature on stress responses in a range of species, there is still much to be learned about the mechanisms of stress responses so that strategies can be developed to prevent and overcome stress-induced disorders. An understanding of the neuroendocrine mechanisms that underlie natural physiological states of stress responsiveness will might provide knowledge that could be utilized to generate physiological treatments for people at risk of illness due to chronic stress and/or disorders of the stress systems. This is conceptually attractive because the most effective mechanisms to suppress stress responses will undoubtedly be those that the body itself uses. Thus to understand nature of stress response and mechanisms by which stress disorder develop, it is necessary to understand how and why stress response evolved.

During the process of evolution single celled organisms w/o nuclei exhibited limited ability to adapt or develop. Dramatic genetic shift accrued in response to the appearance of ‘intones’ which suddenly made innovative adaptive changes in cell structure and function possible. The processes that led to these cell changes were the foundation genetic changes that made possible subsequent brain structure or functions enabling adaptive learning and cognition. These changes made possible the development of complete life support systems (respiration, circulation, digestion, elimination, immune system) enabling the organism to survive in a hostile or challenging stressful environment thus we can say or quote stress as requiring the
ability to learn from and adapt to experience in order to maintain homeostasis. If it had not happened for stress we humans would not have survived the process of evolution. This suggests the ability of living organism to adapt to the massive changes in their surroundings. [59,60]

2.1.1. Historical Development of concept of Stress:

The concept of "homeostasis" goes back to the ancient Greeks. The philosopher Empedocles considered that all matter was a harmonious mixture of elements and qualities [61]. This early expression of homeostasis was extended to living beings by Hippocrates, who considered health as the state of harmonious balance and disease as the state of dysharmony [62]. Hippocrates described disturbing forces of nature as causes of disease and referred to the healing forces inherent to the organism as the "healing power of Nature." This was later called by Galen "vis medicatrix Naturae." Psychogenic stress was mentioned by Epicurus, who suggested that coping with emotional stressors was a way to improve the "quality" of life [63]. In the early nineteenth century, the French physiologist Claude Bernard introduced a theory suggesting that, as organisms become more independent of their surroundings by developing more complex ways of stabilizing their internal environments to counter the changes in their external environment. The importance of adaptive mechanisms was thus recognized [64]. In the early 1900's, Walter Cannon expanded this theory and coined the term "homeostasis." He demonstrated in several experiments that the sympathoadrenal system was responsible for coordinating the "fight or flight" response necessary to meet external challenges [65]. Cannon proposed that there was a "critical" level of stress, in terms of magnitude and duration, against which the homeostatic mechanisms fail and the organism perishes. Cannon believed that an individual organism's susceptibility to this critical stress varies under different general conditions and during the normal and pathologic ups and down of existence in an ordinary life-cycle [65]. In 1936, Selye presented his concept of the "General Adaptation Syndrome," (GAS) and attention shifted from the sympathetic nervous system to the adrenal glands [66-68]. Hams, suggested in 1948 that hypothalamic releasing or inhibiting factors regulate anterior pituitary function [69]. Saffran and Schally demonstrated in 1955 that a factor from the hypothalamus was regulating ACTH release from the pituitary [70]. They named this factor "corticotropin-releasing factor." After intensive investigation, in 1981, Vale and others were able to isolate and characterize its structure as a 41 amino-acid peptide [71]. When the chemical structure was identified, the term "factor" was changed to
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"hormone." Theory in stress biology conceptualized that an integrated "stress system" is consisting of neuroanatomical and functional structures that function to produce the behavioural, physiological and biochemical changes directed toward maintaining homeostasis [72]. This theory is supported by recent findings in neurobiology demonstrating anatomical and functional connections between the hypothalamus, the arousal centre in the pons, and several sympathetic nuclei in the hindbrain [73,74]. In the periphery, the adrenocortical and sympathetic divisions of the stress system have additional integrative actions. These include complementary and permissive interactions of glucocorticoids and catecholamines in the regulation and maintenance of metabolic and cardiovascular homeostasis [75,76].

The term "stress" describes a state of threatened "homeostasis" (Greek for "steady state") or threatened harmony, balance, or equilibrium. The threatening, disturbing forces are defined as "stressors," while the counteracting forces put forth to neutralize the effects of the stressors and re-establish homeostasis are called the "adaptive response" [77].

2.1.3. General Adaptation Syndrome:[59]

Dr. Hans Seyle the world authority on stress defined stress as sum of all nonspecific responses of the body to any demand made up on it, fundamentally it is a physiological response, primary object of which is to maintain life and re-establish the normal state [78,79]. Apart from many specific defence reactions there is an integrated syndrome of closely interrelated adaptive reactions to non-specific stress, this has been termed the General Adaptation syndrome (GAS).

![General Adaptation Syndrome (GAS)](Figure:2.1 1: General Adaptation syndrome)

**General Adaptation Syndrome (GAS)** actually denotes the body’s long-term and short-term response to stress. This specific syndrome depicts a three phase reactions to nervous tension,
such as Alarm reaction stage, resistance stage and adaptation stage with respect to handling stress.

1) An initial "alarm reaction," is characterized by an immediate sympathoadrenomedullary discharge. During the initial alarm stage, the body homeostasis tends to get disturbed owing to stress. Subconsciously the brain recognizes the factor, which causes the stress and accordingly makes the body ready either to flee or to fight back, a reaction at times known as the flight or fight response. As soon as a stressor is recognized by the mind, the cerebral cortex becomes alert unconsciously or consciously. The cerebral cortex senses a danger and instantly activates an automatic nervous system reaction, which makes the body ready for action. Besides this, the hypothalamus, which is a part of the brain helps to regulate and ascertain the response to stress situations in general. As soon as the hypothalamus senses that additional energy is required for combating stress, it rouses the adrenal glands so as to discharge the hormone epinephrine, which is also known as adrenaline. The production of adrenaline makes the body to work immediately. The alarm reaction stage lasts for a short time span, during which people normally feel muscle tensing, dry mouth, high levels of blood pressure and “butterflies” inside the stomach. This stage is only the beginning phase of stimulating the mind and body to effectively cope with the current threat or stress.

The second phase of General Adaptation Syndrome is the resistance stage. During this phase, the body is capable of actively coping with the stressful condition. In case this stage carries on for an extended time frame devoid of any rest and relaxation to balance the stress reaction and permit the body the time to repair and replenish from the effort needed to carry out the correct stress reaction. Resistance stage GAS allows the body to continue facing a stressor after alarm stage, This a long term reaction and is initiated by regulating hormones secreted by the hypothalamus such as corticotropin releasing (CRH) growth hormone releasing hormone (GRH) and thyrotrophic releasing hormone (TRH) CRH stimulates release of ACTH which stimulates adrenal cortex. Mineralocorticoids released causes conservation of Na thus resulting elimination of hydrogen ions whose concentration is build up high due to increasing of body pH. Glucocorticoids produced elevates BP accelerates protein catabolism, glycogenolysis reduce inflammation etc. TRH causes secretion of thyroxine and triiodothyronine which decreased catabolism of carbohydrates GRH causes secretion of human grown hormone which increases body catabolism thus the combined action of CRH
TRH and GRH leads to supply of additional energy to the body to resist the adverse implications of stress.

The third stage of General Adaptation Syndrome is the exhaustion stage. If resistance stage fails to fight against stress then the body moves in to third i.e, stage of exhaustion. An individual is capable of only escaping or fighting so long prior to they start to wear away in their ability to defend against and cope with it. When the stress is excessive and chronic, without any real chance to adapt or recover effectively the stage of exhaustion begins, the individual will start to reveal symptoms of adaptation breakdown. Systems start to collapse and individuals are more vulnerable to various bio-psychosocial symptoms. Unfortunately scientific evidence indicates that we do not adapt positively to chronic stress. That in fact constant exposure to stress facilitates the response to further stressors which compensates for the negative feedback [80]. Thus the constant release of excessive cortisol stops itself from habituating to the stressful events as it would with one time occurrences or normal secretion and continues to be released into the blood. The effects of cortisol create wide spread physiological effects. Chronic activation of the catabolic processes of the stress response can ultimately become destructive and pathogenic. Thus metabolic (myopathy, fatigue, changes in glycemia) and cardiovascular consequences (hypertension), compromised growth and tissue repair, peptic ulceration, reproductive suppression (impotence and amenorrhea), as well as consequences of immunosuppression (increased susceptibility to infection and cancer) can occur when the state of stress is unduly prolonged.

2.1.4. Types of stressors

Stressors include a wide variety of environmental stimuli which evoke significant homeostatic alterations in the host. They may result from natural changes in the environment, or artificial changes, imposed by management constraints, or be caused by a range of experimental stimuli which have been used to study stress physiology. The effect produced by exposure to stress may be influenced by the severity of the stressor, the time during which it is applied (acute vs. chronic), or whether the animal can escape the stressor if it is applied repeatedly (escapable vs. inescapable).

a. Environmental: Natural exposure to extremes of heat or cold under climatic conditions, or artificially in animal housing or holding facilities, are amongst the commonest stressors. Physical restraint associated with separation, treatment or
transport of animals is another important stressor. Environmental changes which produce strange or novel sounds, sights, odours or tastes can also evoke stress. Drugs or chemicals used in management or treatment of animals can act as stressors, while toxic products released by infectious agents, environmental pollutants or inadequate ventilation can have a similar effect. Shearing, docking and castration, carried out during routine management, also cause stress.

b. **Behavioural:** Overcrowding, hierarchical challenge, weaning, exposure to unfamiliar surroundings or isolation can have a major behavioural impact on animals and evoke stress. Changes or restrictions in diet can also act as stressors alone or in combination with stressors such as physical restraint.

c. **Psychological:** Capture of wild animals, or the exposure of domesticated animals to restraint, transport or management extremes may evoke adaptative stress in the host. In the context of Selyes general adaptation syndrome, the response is recognizable initially as anxiety, which may progress to fright or terror. Failure of the animal to express the 'flight-fight' response results in the expression of anger or rage, and if the stimulus persists for a longer period results in frustration or helplessness. Because stress results largely from the individual's perception of the threat posed by the stimulus, rather than its nature per se, a cognitive or psychological component is central to all stress.

**2.1.5. Stress defence Mechanisms**

The HPA axis and the sympathetic system are important regulators of an animal's homeostatic functions [81]. Thus the organism's response to stress is composed of coordination between behavioural, endocrinal, and autonomic components to neutralize the disrupting effects of the "stressors" on homeostasis [82,83].
I. Nervous defence mechanism [84]

Activation of sympathetic branch of the autonomic nervous system causes release of NE in the brain from locus coruleus, (a nucleus deep in the brainstem that projects to many brain areas), amygdala and other limbic structures and frontal cortex. Nervous impulses descend from the hypothalamic vegetative centre, through the autonomic nerves to the peripheral
organs. The splanchnic nerve stimulates adrenal medulla to discharge adrenergic hormones (adrenaline and noradrenalin) in to the blood and other adrenergic nerves influence their target organs directly through fibres. Norepinephrine released causes vasoconstriction and increased cardiac output which raises blood pressure. Gastric secretion is decreased. Released epinephrine stimulates pancreas to release glucagon, decrease insulin and glucose uptake in skeletal muscle and peripheral tissues. Epinephrine is the strongest of catecholamine secreted by stress others include norepinephrine and dopamine. Various effects of catecholamines during stress are:

i. Increased blood flow and glucose metabolism by the brain.
ii. Increase heart rate and force of contraction with peripheral vasoconstriction.
iii. Increased oxygen supply to the lungs and bronchodilation.
iv. Increased glycogenolysis in muscle, increased contraction and increased dilation of skeletal muscle vasculature.
v. Increased glucose production in the liver, increased gluconeogenesis and glycogenolysis with decreased glycogen synthesis
vi. Increased lipolysis, fatty acids and glycerol
vii. Decreased blood flow to the skin
viii. Increased protein breakdown of lymphoid tissue

II. Hormonal defence mechanism: Hypothalamic Pituitary Adrenal axis (HPA axis)

The principal endocrine response to stress is characterized by the so called ‘Shift in anterior lobe hormone production’ which includes; diminished secretion of somatotrophins and gonadotrophins (F.S.H, L.H, prolactin) and thyrotrophin which are not essential for maintenance of life during conditions of emergency. When adaptive capacity of individual is overwhelmed by stressors hypothalamic pituitary adrenal axis is activated. During stress message from central nervous system trigger the hypothalamus to release corticotrophin releasing hormone or factor (CRH or CRF) which stimulates both posterior and anterior pituitary glands causing increase in the secretion of adrenocorticotropic hormone A.C.T.H.
**A]. Corticotrophin releasing hormone (CRH):**

CRH in addition to acting as hormone, acts as neurotransmitter and neuromodulator in the brain. CRH is expressed in amygdala, prefrontal cortex, cingulate cortex. Centrally introduced CRH induces behaviours similar to natural stress (anxiogenic). Thus in a brain it acts as neuromodulator. Since it is released in brain areas important for emotions, there it coordinates behavioural and emotional response to stressors. Effects seen by activation of HPA axis last longer than that of effects seen by activation of sympathetic nervous system and are regarded as the more damaging pathway of chronic stress. CRH is found in neuronal cell bodies in the paraventricular nucleus (PVN) [85,86]. CRH neurons project to the median eminence, where they terminate on the capillaries of the hypophyseal portal vessels. These vessels function as a direct short vascular pathway from the hypothalamus to the anterior pituitary. Thus CRH, which is released into the hypophyseal portal system, is transported to the anterior pituitary, where it stimulates pituitary corticotrophs to both synthesize and secrete ACTH. A different set of PVN CRH neurons send projections to the hindbrain, where they stimulate the electrical activity of their target neurons in the arousal and sympathetic centres.[87].

There are also extra hypothalamic CRH neurons. CRH has been demonstrated in the brainstem, midbrain, striatum, hippocampus, cerebral cortex, spinal cord, sympathetic ganglia and adrenal gland [88,89]. the broad distribution of CRH and its receptors in the CNS provides wide-ranging behavioural effects of this peptide. The CRH receptor is highly concentrated in the brain, anterior pituitary, adrenal medulla and sympathetic ganglia of the
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rat and several primates [90,91]. CRH receptors are coupled to GαS linked G protein complexes CRH binds to either CRH-R1, or CRH -R2 receptors to mediate their functions. Corticotrophic cells express CRH-R1 which when activated stimulates phospho kinase A (PKA) and phosphorylation of calcium channels enables greater calcium influx. Increased calcium influx stimulates exocytosis of the corticotrophic peptide hormones. The corticotrophs are produced by pro-opiomelanocortin (POMC) molecule that is cleaved in to different peptides including adrenocorticotrophin hormone (ACTH)

CRH secretion can be affected by stimuli such as emotion, pain and changes in blood pressure [92]. Several neurotransmitter systems regulate PVN CRH release Both nor epinephrine (NE) and epinephrine (E) stimulate CRH release. It appears that the former stimulates CRH release via the α1adrenergic receptor. Acetylcholine and serotonin are also excitatory mediators participate in both the circadian rhythm and stress-induced release of CRH, whereas gamma-aminobutyric acid (GABA), the opioid peptide system, ACTH, and glucocorticoids are inhibitory [93]. Several products of the immune system such as several cytokines, including Interleukin-1 (IL-1), Interleukin-2 (IL-2), Interleukin-6 (IL-6), or inflammatory mediators, such as platelet activating factor (PAF) and tumour necrosis factor (TNF) appear to stimulate secretion of hypothalamic CRH in vitro and in vivo [94-96]. The distribution of CRH within and beyond the hypothalamus provides an anatomical context for the observation that CRH can simultaneously activate and coordinate metabolic, circulatory and behavioral responses during adaptive situations [97-99].

Experimentally it is observed that ICV injected CRH produces the effects simulating to those observed during stress [100,101]. which includes locomotor activation in familiar environment and freezing behaviour in foreign environment, increased sniffing , grooming and rearing [102]. At higher doses CRH produces bizarre behaviours, including repetitive
locomotion, irritability, or demonstrations of aggression and decrease sexual behaviours [103-105].

**B) Adrenocorticotropic hormone (ACTH)**

ACTH is released targeting the adrenal cortex to secrete cortisol and aldosterone. The ACTH molecule interacts with membrane receptors of the cells of the adrenal cortex and through its second messenger, cyclic AMP, stimulates the rate-limiting step in steroidogenesis (the conversion of cholesterol to pregnenolone) leading to cortisol secretion. The free circulating cortisol acts in a negative-feedback manner to control the release of ACTH from the pituitary gland. Overriding this system of negative-feedback control are the higher centres of the brain, which establish the normal diurnal variation of cortisol. Serum cortisol levels are normally highest in the morning on waking and lowest in the late evening. The circadian pattern of ACTH release is controlled by means of CRH secretion from the hypothalamus. Short-term release of cortisol is episodic, following the pattern of ACTH pulses by about 2 to 3 minutes. Stress is another factor that can override the negative feedback of cortisol on ACTH release. Stress stimulates release of neurogenic amines, which in turn stimulates the release of CRH. Eating and exercise can also influence these pulses. The inflammatory cytokines, tumor necrosis factor α, interleukin-1, and interleukin-6, also stimulate the release of ACTH. ACTH levels can increase up to tenfold in times of stress, resulting in high levels of cortisol. Hypoglycemia, which is a form of chemical stress, can also increase CRH release, ultimately leading to an increase in cortisol. This effect is mediated by glucose receptors in the hypothalamus which stimulate the release of CRH. ACTH is released from corticotrophin cells into the circulation. It binds to its receptors on adrenal cortex mainly in zona fasciculate/reticular. ACTH stimulates the adrenal cortex to produce and secrete glucocorticoids (mineralocorticoids and aldosterone) and weak androgens. ACTH mediates hormone function by binding to receptors called melanocortin – 2 receptor.
Figure 2.1.5: ACTH receptor activation

ACTH binds to its receptors located on adrenal cell membranes activating a Gs-protein resulting in an increase of intracellular cyclic adenosine monophosphate (cAMP). ACTH stimulates cortisol synthesis and secretion by affecting several steps in the steroidogenesis pathway:

ACTH increases the number of low-density lipoprotein (LDL) receptors resulting in increased cholesterol uptake, the precursor for the biosynthesis of all steroid hormones. ACTH stimulates the cleavage of the side-chain of cholesterol converting it to pregnenolone, the first and rate-limiting step in cortisol production. The CYP11A1 gene encodes the cholesterol side-chain cleavage enzyme cytochrome P450. ACTH hydroxylases pregnenolone to give 17-OH-pregnenolone, which then travels to the endoplasmic reticulum for conversion to 11-deoxycorticisol. 11-deoxycorticisol moves back to mitochondria where another hydroxylation takes place at position 21 to produce the final product, cortisol. Cortisol is not stored in the adrenal cortex but is promptly secreted. The adrenal cortex
synthesizes cortisol to maintain its normal serum levels for only few minutes. Thus, the effect of ACTH on adrenal cortisol production can be measured in the serum within minutes from its induction.

C]. Glucocorticoids (GC): Glucocorticoid synthesized in adrenal cortex are released from where they easily enter the cell and interact with a cytosolic glucocorticoid receptors (GR) causing a change in the GR confirmation. The GR is normally chaperoned by HSP 70 and HSP 90 which prevents its translocation in to nucleus Binding of glucocorticoid to their receptors results in conformational change of GR that allows its dissociation from its chaperons subsequently the hormone translocates to the nucleus where it dimerizes and binds to glucocorticoid response elements in the promoter region of various genes causing either inhibition or augmentation of gene transcription. This process requires hours before the response is seen. Evidence suggests that membrane receptors may also mediate the some glucocorticoid effects as well.

Glucocorticoid receptors are present throughout the brain, including in the CRH neurons of the hypothalamus[106]. The actions of glucocorticoids on the central nervous system (CNS) are mediated by two separate receptor systems: glucocorticoid receptors type I and type II [107,108].

![Figure 2.1.6: Mechanism Of Action of Glucocorticoids](image)
Type I ("corticosterone receptors") receptors are found mainly in the neurons of the limbic structures, such as the hippocampus and septum [109]. These receptors play a role in modulating the response to environmental and emotional stimuli, with consequent changes in behavior and HPA axis activity. Type I receptors have a high affinity for the primary glucocorticoid (cortisol/corticosterone), and are similar to "mineralocorticoid receptors" of the kidney [107,108]. In the limbic system, these receptors have a high specificity for corticosterone as an agonist, whereas the mineralocorticoid aldosterone appears to be a competitive antagonist. The Type I receptors that are found in the circumventricular organs function as mineralocorticoid receptors that respond to aldosterone and act to regulate sodium homeostasis, cardiovascular control and salt appetite [108]. With age, the hippocampus loses approximately 50 percent of its glucocorticoid type I binding sites [110,111].

Type II glucocorticoid receptors are present at high concentrations in the hypothalamus, particularly in the CRH neurons. Type II receptors also are found in the brain areas that contain POMC, such as the hippocampus, lateral septum, amygdala, and nucleus tractus solitarius [112]. At these sites, it is likely that the receptors participate in the behavioral, neuroendocrine and autonomic responses to stress [108]. During stress, the occupancy of type I receptor changes only minimally, whereas that of type II receptor changes considerably [107]. Glucocorticoids exert negative feedback to terminate ACTH release in response to stress and have long-term effects on adaptive behaviours, presumably via the type II receptors. Type II receptors diminish with age, but are not affected by ACTH [113,114]. Reduction of type II receptors is associated with decreases of the negative feedback action of glucocorticoids, which may result in a more persistent elevation of circulating plasma corticosteroid levels following stress [115,116]. In addition, persistent elevations of circulating glucocorticoids render the neurons of the hippocampus vulnerable to toxic influences with consequent degeneration and death of the cells [117].
The main effects of corticosteroids on the human body are as follows:-

I) **Metabolism** :-

a) **Carbohydrate metabolism** :- During stress corticosteroids provide protection of glucose dependent tissue (eg heart and brain) from starvation. It exerts this effect by stimulating liver to form glucose from amino acids and glycerol and by stimulating glucose deposition as liver glycogen. This is achieved by increase breakdown of proteins and stimulation of lipolysis to librate amino acids glycerol respectively for gluconeogenesis. This effect combined with diminished peripheral utilisation of glucose results in increased blood glucose levels. The mechanism by which these actions develop are not fully recognized but decreased glucose utilisation is due to translocation of glucose transport proteins from the cell membranes to some intracellular location in various tissues. This results in many side effects observed with long term glucocorticoid increase such as atrophy of lymphoid tissue, decreased muscle mass and thinning of the skin.

b) **Protein metabolism** :- It increases protein synthesis in the liver increase in plasma level of amino acids, stimulates deamination in the liver and decreases protein synthesis in muscle, lymphoid tissue, adipose tissue, skin and bone.

c) **Lipid metabolism** :- Adipocytes in the trunk region respond preferentially to elevated insulin levels in response to glucocorticoid induced elevation of blood glucose since insulin stimulates glucose uptake this can result in fat deposition in the truncal region Adipocytes in the periphery respond preferentially to glucocorticoides facilitated effects of their lipolytic hormones i.e, permissive effect-of cortical, fat is
redistributed to truncal regions while peripheral extremities lose fat. Thus long term increases in cortisol result in abdominal obesity combined with emaciated limbs.

II) Effect on Immunity and blood: It acts up on blood. Decreases circulation eosinophils (eosinopenia) lymphocytes (lymphopenia) and monocytes and polymorphonuclear leucytosoi. The contraction of spleen discharges its storage blood in to the general circulation RBC production is accelerated. Decreased accumulation of leukocytes at the site of inflammation delays healing, inhibits fever, decreases tissue mass of lymphid tissue and blocks cell mediated immunity.

III) Electrolyte and water balance: Glucocorticoids can stimulate renal sodium reabsorption and potassium and hydrogen loss through renal tubule. This process helps to retain sodium and thus increased blood volume.

IV) Antinflammatory effects: cortical provides a mechanism to control excessive stimulation of the inflammatory response initiated by stress 5-a injury and disease. They also inhibit the production of fibroblast. which develops connective tissue cells. Injured fibroblasts release chemicals that play a role in stimulating inflammatory response thus glucocorticoid reduce. Inflammation but at the same time they discourage connective tissue formation by fibroblasts thus would healing is slow during stress.

V) Immune suppressive

Immune responses are also inhibited by glucocorticoid. The inhibition occurs at many levels of immune system. Prostaglandins and leukotriene production is inhibited by glucocortid . Induction of lipocortin inhibits phospholipids A2 in macrophages and monocytes. Cytokines IL – 1 , IL -6 and TNF – L expressions are inhibited in macrophages monocytes and lymphocytes. Glucocorticoids suppress growth factor induced DNA synthesis and fibroblast proliferation.

V) Other actions include

Enhanced gastric secretion juicy excretion, Decreased bone formation

VII) Blood pressure Glucocortioids make blood vessels more sensitive to stimuli that bring about their contraction carting rise in blood pressure. Glucocorticoid leads to sodium retention which further leads to water retentions thus marinating high blood pressure.
2.1.6. Effect of stress on various physiological functions of the body:

I). Effect on Psyche:

It has been proposed that a critical factor in the pathophysiology of several psychiatric syndromes, such as major depression, anorexia nervosa and panic anxiety, stems from an abnormality in the counter regulation of the generalized stress response, resulting in CRH and/or central catecholamine hypersecretion [118]. In particular, it has been hypothesized that abnormalities in the positive regulation or defects in counter regulation of the central components of the adrenocortical and adrenergic system are responsible for these disorders [119,120]. The association between stress and depression stem from several observations. Depression-prone individuals have a higher than expected incidence of early noxious stress associated with major life changes. Acute stress-induced hormonal and behavioural changes closely resemble the symptom complex of depression [121]. Hypercortisolism is a consistent feature of the classic form of major depression [122]. Melancholia. It is an organized state of anxiety, resulting in a profound sense of worthlessness and hopelessness about the future. This anxiety about self and the future are associated with other signs of hyperarousal or activation of the generalized stress response which include enhanced vigilance, as well as inhibition of vegetative functions such as feeding, growth, reproduction, and sleep [119,120]. The pituitary corticotroph cells in major depression are restrained by high circulating glucocorticoids. Hypercortisolism in major depression reflects a defect at or above the hypothalamus resulting in the hypersecretion of endogenous CRH [119,120][123,124][128,129].

LC-NE system , is also activated in melancholia. Hence, patients with melancholia show elevated levels of NE in CSF and plasma. Weisset al () have shown that inescapable shock produces a syndrome in the rat that is very analogous to that of melancholia observed in organisms being overwhelmed by stress [123,124][127,128][132,133].

Although there has been a general emphasis on the role of the aminergic systems in stress and depression, there is a evidence that suggests that super sensitivity of central muscarinic mechanisms may be involved in the pathophysiology of depressive disorders [125-129][129-133. Stress results in the rapid activation of the septo hippocampal cholinergic system characterized by an increase uptake of choline and the release of acetylcholine (ACh). The latter has been shown to simultaneously induce alterations in behavioral, cardiovascular and neuroendocrine function characteristic of those observed during stress [125,126,130,131][129,130, 134,135]. Hence, it has been hypothesized that stress-induced
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changes in affective, neuroendocrine, sleep and heart rate profiles may reflect a central muscarinic cholinergic component. In this regard, in vivo and in vitro data suggest that the muscarinic cholinergic agonist arecoline stimulates the HPA axis and this effect is mediated mainly by the release of endogenous CRH [132] [136]. In addition, it appears that the functional activity of ACh and the secretion of hypothalamic CRH are increased in affective disorders.[125,126,133,134] [129,130,137,138]

II). Effect on Growth

In humans, linear growth and final adult stature depend on multiple factors. These include genetic constitution [135][139], nutrition [136] [140] systemic disease [137,138] [141, 142], hormones [139] [143] and psychosocial environment [140] [144]. A stressful psychosocial environment subsequently affects physical stature, intellectual and behavioral development.[141,142] [145,146]. Psychosocial dwarfism, known also as abuse dwarfism, is a human condition occurring due to withdrawal of normal "care" by the caregiver, which may act as an emotional stressor [140] [144]. This syndrome is characterized by three primary reversible impairments which are delayed physical maturation, retardation of intellectual age, delayed social maturation [143,144] [147,148].

It is a commonly hypothesized that glucocorticoids and/or opioids secreted in response to chronic stress inhibit pituitary GH release at the pituitary level and decrease target tissue sensitivity to growth hormone, somatomedin-C or other growth factors. It has been observed that the central administration of CRH decreases GH secretion in rats which suggests a possible role of endogenous CRH in the modulation of GH secretion during stress [145,146] [149,150].

III). Effect on Immunity

The principal effectors of the stress response exert multiple, complex effects on the immunocompetence during stress. The HPA axis can influence immunologic function through a variety of mechanisms, including CRH-mediated actions on the release of somatostatin, with subsequent inhibition of growth hormone, CRH-mediated release of ACTH and beta-endorphin, and CRH mediated pituitary-adrenal activation [147,148] [151,152]. The best understood and most widely studied immunologic effects of the HPA axis are those mediated by glucocorticoids [149,150] [153,154].

Glucocorticoids generally exert immunosuppressive and antiinflammatory effects [149,150] [153,154]. These include inhibition of leukocyte traffic, interference with cell-mediated immunity, and enhancement of suppressor T-cell function[150,151-156] [154,155-160]. Moreover, there is a systematic decrease in the production of cytokines and interference with
their functional effects, as well as the induction of lymphopenia, thymic involution, and loss of splenic and lymph node tissue mass [149,153,155]. It should be noted that, in some instances, glucocorticoids enhance certain components of the immune response, including the function of specific differentiated clones of lymphocytes [161]. The CRH neuron of the HPA axis has recently been shown to participate in a negative feedback loop, producing pituitary adrenal activation and concomitant glucocorticoid-mediated immunosuppression in response to peripheral mediators of the inflammatory response, such as IFN, IL-I, IL-2, and PAF. [150,158,159] [154,162,163]. Interruption of this feedback loop by the administration of glucocorticoid antagonists or an endogenous deficiency in the responsiveness of the CRH neuron to a variety of immune mediators results in susceptibility to inflammatory disease. It has been suggested that patients with major depression show immunosuppression as a consequence of the hypercortisolism [160] [164]. NE is thought to have a variety of effects on the immunologic response [157] [161]. As an example, the spleen and lymph nodes are replete with noradrenergic terminals and adrenergic receptors that modulate the functional activity of these lymphoid organs. A putative bidirectional regulatory feedback loop exists between the immune system and the CRH system. Cytokines and inflammatory mediators stimulate the HPA axis primarily by causing secretion of CRH. The HPA axis, in turn, inhibits the immune/inflammatory response primarily via increases in glucocorticoid secretion. This glucocorticoid-mediated immunosuppression could prevent excessive inflammatory/immune responses during acute stress.

IV). Effect on Reproductive system:

The state of threatened homeostasis produced by physical or emotional stress has long been recognized as a profound disruptive factor in reproductive function. Females under stress may demonstrate delayed puberty, lack of behavioral receptivity, failure of ovulation or embryo implantation, spontaneous abortion, or increased infant mortality [161-164] [165-168]. Males may exhibit suppression of testosterone secretion, spermatogenesis and libido [165-168] [169-172]. The severe suppression of reproduction during stress appears to be caused by several hormones secreted during stress such as CRH, ACTH, beta-endorphin and glucocorticoids on hypothalamic-pituitary-adrenal (HPA) axis function [169-174] [173-178]. Although the mechanism of these effects on reproductive function are not fully elucidated, possible sites involved include:

1) A centrally mediated inhibition of gonadotropin-releasing hormone (GnRH) release by CRH, opioids and glucocorticoids [170,171] [174,175]
2) Glucocorticoid-mediated decrease in pituitary responsiveness to GnRH, resulting in decreased luteinizing hormone (LH) secretion [175] [179] 

3) Direct gonadal effects of glucocorticoids with subsequent alterations in sex steroid output [176,177] [180,181]

Stress- decreases circulating LH and sex steroid levels which might be due to centrally mediated inhibition of GnRH secretion by hormones secreted during stress., resulting in decreased sexual behaviors in males and lack of behavioral receptiveness and complete ovarian inactivity in females [178,179][182,183]. Glucocorticoids suppress GnRH and gonadotropin secretion at both the hypothalamic and pituitary levels [180,181] [184,185]. At the gonadal level, glucocorticoids have a direct inhibitory effect on testicular Leydig cell function. Leydig cell sensitivity to LH and hCG is decreased by glucocorticoids, probably due to glucocorticoid induced reductions in testicular LH receptors [176,177] [180,181]. In menstruating females, glucocorticoid treatment decreased estradiol concentrations and caused resistance of the uterus to estradiol. By decreasing estradiol receptors in the uterus [182] [186] . Stress cannot only influence the ability of the female to conceive, but also can adversely affect the fecundity of each conception. In rodents, stress during pregnancy has been shown to result in smaller litter sizes [183,184] [187,188]. In sheep, there also is evidence that stress and hypercortisolism are associated with increased embryo loss following implantation and may precipitate premature labor [185,186,187]. [189,190,191].

V) .Effect on sleep:

Sleep is believed to be a neural state during which consolidation of declarative memories are taking place [188] [192]. Stress due to Sleep deprivation increases the homeostatic drive to sleep, with resulting changes in proinflammatory cytokines and glycogen levels. The long-term consequences of sleep deprivation constitute a form of allostatic load—-with consequences involving hypertension, reduced parasympathetic tone, increased proinflammatory cytokines, increased oxidative stress, and increased evening cortisol and insulin. In addition reduced sleep is associated with increased risk for obesity, which means increased chances of cardiovascular disease and diabetes. With diabetes there is also poorer cognitive function, as well as increased depression [189] [193].and increased risk for Alzheimer’s disease [190,191] [194,195].

Depressive illness is almost universally associated with disturbed sleep [192] [196] . Thus, there are linkages not only between the multiple, interacting mediators that are involved in allostasis and allostatic load, but also overlaps i.e. co morbidities, between the disorders, such as diabetes, hypertension, cardiovascular disease, and depression that are associated with
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excessive stress and with the deregulation of the systems that normally promote allostasis, or adaptation.

VI). Effect on Memory

A number of studies have reported that the induction of (long term potentiation) LTP in the hippocampus is blocked by the administration of corticosterone [193,194,195] [197,198,199]. Bennett and collaborators [196] [200] reported the existence of a negative correlation between the magnitude of LTP in the CA1 in the hippocampus and the level of circulating corticosteroids, thus suggesting a dose-dependent relationship between corticosteroids and their detrimental effects on LTP. Diamond and collaborators [197] [201] reported the presence of an inverted-U shape relationship between the level of circulating corticosteroids, and LTP. They described a positive correlation between corticosterone and primed burst potentiation (PBP; which is a low threshold form of LTP [196] [200] at low levels of corticosteroids and a negative correlation between corticosterone and PBP at high levels of corticosteroids. These results provided a strong support for the hypothesis that corticosteroids exert a concentration-dependent biphasic influence on LTP. Modulatory effects of corticosteroids on animal cognition have been reported For example, acute administration of either corticosterone or dexamethasone accelerates the rate of extinction of a shock avoidance response [198,199,200,201]. In 1976, Kovacs and collaborators [202] reported that low doses of corticosterone facilitate extinction of an avoidance response, while high doses of corticosterone delay the rate of extinction of the conditioned response [203] [207].

Altogether, these results confirmed the important role of the hippocampus in explaining corticosteroid-induced cognitive impair. This dissociation between the effects of basal and stress levels of this hormone is not surprising as there are two types of receptors for glucocorticoids Type I, also known as the mineralocorticoid receptors, and Type II, also called the glucocorticoid receptors, and the hippocampus is one of the few sites in the body in which there are substantial concentrations of both types of receptors [204] [208]. The high-affinity Type I receptors are heavily occupied by basal levels of adrenal steroids during the diurnal cycle, whereas elevation of glucocorticoid concentrations during stress increases the occupation of the lower-affinity Type II receptors. Thus, this provides an ideal mechanism by which circulating adrenal steroids can have different, or even opposing, effects upon the hippocampus during basal versus stress conditions [205] [209]. Low levels of endogenous corticosterone by occupying Type I receptors enhance PBP [206] [210]. whereas occupation
of Type II receptors by high levels of endogenous glucocorticoids suppresses LTP [197] and PBP [197,208].

Glucocorticoids (GC) inhibit the removal of glutamate from the synaptic cleft at glia, and are speculated to occur at neurons as well. As a result of these GC actions, there is enhanced mobilization of free cytosolic calcium in the postsynaptic neuron. This accumulation of calcium is augmented by inhibiting the efflux of calcium via both the Ca2+/ATPase and the Ca2+/Na+ exchanger. Due to excessive cytosolic calcium, GC exacerbate calcium-dependent degenerative events which include worsening the proteolysis of the cytoskeletal protein spectrin, the accumulation of the abnormally phosphorylated tau protein, and the production of oxygen radicals during necrotic insults[208] leading to the neuronal damage in hippocampus which might be causing detrimental effects on memory.

A third mechanism by which stress might impact the cellular mechanism of memory formation is via glucocorticoid inhibition of glucose transport in the hippocampus, as assessed by 2-deoxyglucose mapping in vivo [209-212]. Furthermore, glucocorticoids inhibit glucose uptake into both cultured hippocampal neurons and glia [213,214]. These disruptive glucocorticoid actions are mediated by the Type II receptor [215] and appeared to involve both translocation of pre-existing glucose transporters molecules from the cell surface to intracellular storage sites [216] as well as decrease in levels of mRNA for the glucose transporters [217].
2.2. Anti stress drugs (adaptogens)

The concept "Adaptogen" was coined in 1947 by the Russian scientist, Lazarev [27]. He discovered the adaptogenic effect of dibasol (2-benzylbenzimidazol) in tests aimed at the stimulation of non-specific powers of resistance in test subjects. Lazarev, who called this new group of medically-effective substances, "adaptogens," defined them as substances meant to put the organism into a state of non-specific heightened resistance in order to better resist stresses and adapt to extraordinary challenges. It was Selye who examined the actions and consequences of such stresses on the healthy organism [218]. He formulated the "General Adaptive Syndrome" (GAS) as a consistent, non-specific response of the organism to stressful influences of totally diverse types, the adaptive reaction enables the body to heighten its power of resistance towards stresses, and to adapt to external conditions. The limiting factor within this adaptive capacity is, according to Seyle, determined by the so-called "Adaptations Energy" [219] of the organism. This means that the resistance reserves towards unfavourable influences are not inexhaustible, but they diminish by extreme stressfulness. The consequences are misadaptation and diseases. Brekhman, who examined the effects of adaptogenic drugs at a later point, summarized the concept "adaptogen" in 1958, as follows [220].

1. It must show a non-specific effect (raising the power of resistance to toxins of a physical, chemical or biological nature).

2. It is to normalize, independent of the type of pathological condition

3. It must be harmless and disturb the body functions as little as possible.

Accordingly, adaptogens are to strengthen the non-specific powers of resistance to non-infectious stresses, raise the general performance capacity during stress situations and thereby prevent diseases that could develop due to over-stressing the organism.

2.2.1. Adaptogens Definition and Differentiation from other Drugs with Related Pharmacological Effects

If one accepts the concept of adaptogenic effects in the medical sense, it is necessary to define and differentiate them from other remedies of related action. Although a strict differentiation is not possible, there is a number of criteria which allow a formal arrangement of these other drugs in immune stimulants, Nootropics, anabolics, tonics and geriatric aids.
I). **Adaptogens** were originally defined as drugs enhancing the “state of non-specific resistance” in stress [221,31]. This definition implies that an organism has different levels of resistance to stress associated with the activities of the nervous (CNS and sympathetic) and endocrine [hypothalamuspituitary- adrenal (HPA) axis] systems and with innate immunity, i.e. the activity of the non-specific immune system (antimicrobial enzyme system, non-specific cytokines, complement system, phagocytic cells and natural killer cells). However, this representation is related to a physiological condition, i.e. stress, but not to a specific illness. Later on Brekhman and Dardimov [33] postulated that:

(i) adaptogens must reduce stress-induced damage, thus presenting stress-protective effects such as anti-fatigue, antiinfectious, anti-depressant and restorative activities;

(ii) adaptogens must exhibit stimulating effects, both after single and multiple administration, leading to increased working capacity and mental performance against a background of fatigue and stress;

(iii) the stimulating effect of adaptogens must be different from those of conventional CNS stimulants and anabolics that deplete the energetic and plastic resources of the organism and give rise to negative side effects such as drug withdrawal syndrome; and

adaptogens must be innocuous and must not perturb body functions from their normal levels but rather exert a normalising influence on a pathological state, independent of the nature of that state.

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Perspectives</th>
<th>Term</th>
<th>Description of effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ayurvedic</td>
<td>Rasayana</td>
<td>Rejuvenates</td>
</tr>
<tr>
<td>2.</td>
<td>Traditional Chinese</td>
<td>Qi tonic Superior herb</td>
<td>Strengthens and stimulates the immune and defence functions of body</td>
</tr>
<tr>
<td>3.</td>
<td>Russian Scientist</td>
<td>Adaptogens</td>
<td>Increase resistance within body to a wide range of stressors and normalizes functions</td>
</tr>
<tr>
<td>4.</td>
<td>Western scientist</td>
<td>Adaptogens</td>
<td>Regulates the HPA axis and sympatho adrenal system</td>
</tr>
<tr>
<td>5.</td>
<td>Clinical Herbalist</td>
<td>Adaptogens</td>
<td>Reregulat es disharmony in the neuroendocrine and immune system</td>
</tr>
</tbody>
</table>

**Table 2.2.1: Summary of different perspectives [222]**

II.) **Immune Stimulants** are substances which bring about a heightened resistance through the stimulation of non-specific defensive processes which are largely independent of antigens
There occurs a rise in non-specific resistance towards bacterial, and especially viral infections, as also in chronic inflammation.

III) **Nootropics** (cognition enhancers), according to Giurgea [224] are effective psychopharmacological agents which are said to improve the higher integrative brain functions, such as memory, learning, understanding, thinking and the capacity for concentration. No specific mechanism is known. It is assumed that nootropics stimulate existing neural synapses to optimum performance (adaptive capacity), and also for damaging influences, such as disturbances of the energy and neurotransmitter metabolism or ischemia (protective capacity).

IV.) **Anabolics** are substances which activate the anabolic metabolism. They promote the synthesis of nucleic acids and protein metabolism; thereby in general, growth. No precise conceptual definition can be given for tonics and geriatric remedies. They fall into the category of wellness enhancers and are therefore without pharmacological significance.

V.) **Tonics**, according to a very generalized definition, are substances which mitigate conditions of weakness or lack of tone within the entire organism, or in particular organs.

Tonic herbs are important in chineese medicines in which their main function is to supplement deficiencies and enhance energy and well being. Adaptogens raise one's capacity, therefore may also be included in the group of "tonics."

VI.) **Geriatric remedies** are substances serving as a preventative treatment of "old-age diseases." "Stiffness and age-conditioned rigidity, are possibly the outer manifestations of diminished or lacking ability to adapt.

VII.) **Stimulants**, defined as drugs that increase the activity of the sympathetic nervous system, produce a sense of euphoria and can be used to increase alertness and the ability to concentrate on mental tasks. Stimulants such as caffeine, nicotine, amphetamines and cocaine, are also used, and sometimes abused, to boost endurance and productivity. However, long-term stimulant abuse can impair mental function and lead to psychotic symptoms. Furthermore, traditional stimulants that possess addiction, tolerance and abuse potential, produce a negative effect on sleep structure, and cause rebound hypersomnolence or "come down" effects. By definition, plant adaptogens do not exhibit such negative effects: in fact one plant adaptogen, that derived from *Rhodiola rosea*, has been shown significantly to regulate high-altitude sleep disorders and to improve sleep quality [225]. Plant adaptogens
stimulate the nervous system by mechanisms that are totally different from those of traditional stimulants, being associated rather with metabolic regulation of various elements of the stress-system and modulation of stimulus-response coupling[226-229,34,32].

Depending on the mediators of the stress system involved in the adaptogen induced stress-response, an immediate (single dose effect) or a long term (after multiple administration) stimulating effect may be observed. It is seen as characteristic of adaptogens that their anti-stress effect towards stresses of a non-infectious variety, always stands in the foreground. Although in so-called adaptogens," CNS stimulant , immune-stimulating, nootropic, or metabolic effects have also been observed.

<table>
<thead>
<tr>
<th></th>
<th>Stimulants</th>
<th>Adaptogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery after exhaustive physical loading</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Energy depletion</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Performance under stress</td>
<td>Decrease</td>
<td>Increase</td>
</tr>
<tr>
<td>Survival under stress</td>
<td>Decrease</td>
<td>Increase</td>
</tr>
<tr>
<td>Insomnia</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Side effects</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>DNA/RNA and protein synthesis</td>
<td>Decrease</td>
<td>Increase</td>
</tr>
</tbody>
</table>

*Table 2.2.2:Differences Between Stimulants and Adaptogens*

### 2.2.2.Structure activity relationhip of adaptogens:

In terms of active ingredients, Antistress preparations can be divided into the three groups of:

1. those that contain phenolic compound such as phenylpropanoids, phenylethane derivatives, and lignans, [230-233,34] whose structural resemblance to catecholamines could suggest an effect on the sympathoadrenal system and possibly imply an effect in the early stages of the stress response;

2. those that contain tetracyclic triterpenes [233,38] such as cucurbitacin R diglucoside,[235,236].which structurally resemble the specific corticosteroids that inactivate the stress system to protect against overreaction to stressors.

3. oxylipins—unsaturated trihydroxy or epoxy fatty acids structurally similar to leukotrienes and lipoxines. [237,238]. The first group of adaptogenic extracts named above
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includes the roots and rhizome of *E.senticosus* and *R.rosea*, as well as extracts of *S.chinensis* fruits. The second group of adaptogenic substances are contained in extracts of *B. alba* and *W. somnifera*. The third group of adaptogenic compounds have been found in *B.alba* and *G.glabra*. Anti stress agents apparently increase the ability of The stress system to respond to stress stimuli in a manner that tends to preserve homeostasis, particularly by modulating the biosynthesis of eicosanoids including prostaglandins E2 and F2,5-hydroxyeicosatetraenoic acid (5-HETE), 12-HETE, and Leukotriene B4. Moreover, adaptogens also appear to regulate the Basal level of the arachidonic acid which is a precursor and are observed to do this under various stressful conditions, such as immobilization, heavy physical exercise, and radiation injury [237,239-242]. Although there is a difference in the mode of action and pharmacological activity of different adaptogens ,[2434246], it is difficult to relate these in a satisfactory way to the differences in adaptogens’ various effects. However, the mechanisms of action of adaptogens are mainly related to effects on the neuroendocrine- immunologic axis that constitutes the stress system [245-248].

2.2.3. Phytoconstituents contributing to antistress effect:
Various active constituents found in herbal adaptogens work to stimulate neuroendocrine and immune systems via multiple metabolic pathways. They affect the brain nerves, endocrine glands (pituitary, thyroid, parathyroid, adrenal, thymus, pineal pancreas, ovaries and testes) and immune system by helping to re-regulate normalise and enhance functions.

Key constituents of *Eleutherococcus* root include the eleutherosides, triterpenoid saponins and glycans [47]. The leaf of Siberian ginseng contains hyperoside, a flavonol glycoside, which showed remarkable antistress activity in a forced swimming test on mice [249]. Isofraxidin in the bark and hyperoside in the leaf are respectively reported as effective sedative components. Rhodiola also had the highest polyphenol content which may not only have adaptogen properties but may decrease the risk of complications induced by oxidative stress [250]. Withania has immunomodulatory, anti-inflammatory but most significantly adaptogenic effects, which may result from the complex of the many steroidal withanolides found in the root of the herb [251].
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<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Adaptogen</th>
<th>Active ingredients</th>
<th>References</th>
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<tr>
<td>1.</td>
<td>American ginseng</td>
<td>Ginsenoside</td>
<td>252</td>
</tr>
<tr>
<td>2.</td>
<td>Asian Ginseng</td>
<td>Ginsenoside</td>
<td>253</td>
</tr>
<tr>
<td>3.</td>
<td>Ashwgandha (Indian ginseng)</td>
<td>Withanolides, Sitoindosides withaferin, somniferin, Withanin, Anaferine</td>
<td>254</td>
</tr>
<tr>
<td>4.</td>
<td>Rhodiola</td>
<td>Rosavin (rosavin, rosin, rosarin), Salidrose, flavonoids</td>
<td>234, 240</td>
</tr>
<tr>
<td>5.</td>
<td>Eleuthrococcus</td>
<td>Eleutheroside</td>
<td>259, 268</td>
</tr>
<tr>
<td>6.</td>
<td>Amlal</td>
<td>Ellagic acid, Phyllemblin, quercetin, emblicol,</td>
<td>229, 269</td>
</tr>
<tr>
<td>7.</td>
<td>Astragalus</td>
<td>Astragalons, glucoronic acid, astagulosides, flavones, isoflavones</td>
<td>260, 261, 270, 271</td>
</tr>
<tr>
<td>8.</td>
<td>Shilajit</td>
<td>Humic acid, dibenzo alpha pyrones biphenals</td>
<td>262, 272</td>
</tr>
</tbody>
</table>

Table 2.2.3: Major known Phyto constituents in plants with antistress effect

2.2.4. Targets for the actions of antistress drugs:

The primary site of action of drugs with antistress activity appears to be the HPA, and their secondary sites of action appears to be metabolism, liver, components of the immune and cardiovascular systems. The effects of adaptogens become some what more clear when it is recalled that stress is a defensive response to external factors, and that it stimulates the formation of endogenous “messenger” substances such as catecholamines, prostaglandins, cytokines, nitric oxide (NO), and platelet-activating factor, which in turn activate other factors that may either counteract stress or, conversely, induce or facilitate disease. According to this concept, the “stress-executing” or “switch-on” mechanism activates the sympathoadrenal system (SAS) and over the longer term also activates the HPA, together with various regulators of cell and organ function. Counteracting this is the “switch-off” system, which protects cells and organ systems, and thus the entire organism, from damaging overreaction to stress. This switch-off system includes antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase; interleukins that down regulate various aspects of the immune response; certain corticosteroids and eicosanoids such as prostaglandin E2; and antiinflammatory mediators. According to this concept, drugs with antistress activity can be described as agents that reduce the reactivity of the host-defence system to various
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stressors by helping to restore normal homeostasis.[243]. The mechanism of the anti-stress activity of many claimed adaptogens is associated with their effects on:

(i) The mediators of stress response, and especially on the formation of cortisol, nitric oxide (NO), phosphorylated stress-activated protein kinase (p-SAPK)/c-Jun N-terminal protein kinase (p-JNK) [229,260] and Fork head Box O proteins, such as DAF-16 as observed in Caenorhabditis elegans [261]

(ii) The expression of heat shock proteins Hsp70 and Hsp16, which are molecular chaperons involved in stress-induced cytoprotection and in adaptation to repeated exposure to an initial stressor [262,263].

(iii) The biosynthesis of ATP, thus inducing an alteration in energy source [264]. It has been demonstrated that activation of stress response results primarily due to increased expression of NO, Hsp, p-SAPK/p-JNK and cortisol [229,260]. The formation of NO can strongly inhibit the production of cellular energy (ATP) in stress by following two mechanisms:

(a) the inhibition of mitochondrial respiration by the reversible inhibition of cytochrome P450, associated with constitutive isoforms of NO synthase (NOS), or by the irreversible inhibition of P450, associated with inducible NOS (i-NOS) [265].

(b) the inhibition of glycolysis by modification of the SH groups of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme involved in ATP production [266]. In this context, drugs with antistress effect prevent the stress-induced increase in NO and the associated decline in ATP production, thus resulting in increased performance and endurance [231,269].

Cortisol is a stress-hormone involved in the feed-back regulation of stress responses that help to restore homeostasis. Stress produces characteristic changes in the HPA axis, including an increase in cortisol, and reduced sensitivity of the HPA to feedback down-regulation. Increased levels of cortisol in the serum and saliva have been observed in association with physical exertion and various stressors. In chronic stress, prolonged secretion of cortisol
causes muscle wastage, hyperglycemia, and suppresses immune/inflammatory responses [268,269] cognitive functions and, possibly, longevity. Typically, a cell is either in balance (dynamic equilibrium - homeostasis) (Figure a), or functioning under stressful conditions (threatened homeostasis – imbalance), (Figure b). Two further states are possible, namely, that in which the cell is adapted (tolerant) to stress (i.e. state of non-specific resistance to stress; (Figure: c), or the state of apoptosis (dying).

Figure 2.2.2: Normally functioning cell

Figure (2.2.2) shows that mitochondria generate aggressive oxygen-containing radicals that can damage native or repaired proteins by distorting their 3-D structure so that they can no longer fulfil their functions in the cell. There are many other factors involved in the regulation of homeostasis at both the cellular level and the whole organism level. Amongst these are:

i) the stress hormone cortisol (a molecule that is secreted from glands and regulates the functions of organs and systems of the organism) ; glucocorticoid receptors that modulate/regulate cortisol secretion (feedback regulation);

ii) NO, an intracellular signalling molecule that mediates stress response and modulates stress-induced activation of hormonal, nervous and immune systems;
iii) FoxO, a Forkhead protein that controls the synthesis of proteins involved in stress resistance, cell survival and longevity.

Figure 2.2.3: Cell under stress

Figure (2.2.3) shows that in stress (infection, cold, heat, radiation, physical load, emotional stress, etc) an external stress signal activates a cascade of “signalling” proteins/enzymes including JNK, a stress-activated enzyme that plays important roles in the regulation of a diverse array of cellular functions such as neuronal development, activation of the immune system and programmed cell death (apoptosis).

In particular, JNK:- increases the formation of aggressive radicals and nitric oxide, which in turn suppresses the generation of energy providing molecules ATP. As a result of lack of energy, many proteins cannot work, many functions are suppressed, and the first symptoms of fatigue and exhaustion are observed. ATP is also required for the normal functioning of heat shock proteins such as Hsp70, which are produced as a defence response to stress and assist in the repair of misfolded and damaged proteins; suppresses glucocorticoid receptors (GR) such that the feedback inhibition of cortisol secretion ceases to function and levels of circulatory cortisol increase. Cortisol inhibits the immune system, has anti-inflammatory effects on the body and is required in order to protect the organism from over-
reaction/over-activation in response to stress. However, chronically high levels of cortisol are associated with depression, chronic fatigue and impaired cognitive function, including decreased attention and learning ability; 

Figure 2.2.4: Adapted cell with increased resistance to cell 

Figure (2.2.4) shows that antistress agents decrease NO, cortisol and JNK in stress and stimulate/activate the expression of Hsp70 and p-FoxO1. For example ADAPT-232 forte is a proprietary name of a fixed combination of three genuine extracts of *Eleutherococcus senticoccus* (Rupr. et Maxim) Harms root, *Schisandra chinensis* (Turzc) Baill., root, *Rhodiola rosea* L., root and vitaminB5 The stimulation of Hsp70 biosynthesis is a key point in the mechanism of action of drugs with anti stress effect since the heat shock protein enhances the repair of damaged proteins; inhibits the stress-induced expression of NO genes and, since the reduced levels of NO can not suppress the formation of energy providing molecules. ATP is increased to normal levels in the adapted cell; inhibits JNK and consequently apoptotic death and suppression of immune system via activation of glucocorticoid receptors (GR) and other mechanisms. Normal GR function and normal ATP levels are associated with the anti-fatigue and anti-depressive effects of adaptogens along with normal cognitive function (good
During stress molecular mechanisms by which NO formation can strongly inhibit the production of cellular energy are namely by the inhibition of mitochondrial respiration by reversible (from constitutive isoforms of (NOS) and irreversible (from iNOS) inhibition of cytochrome P450 [265] and by inhibition of glycolysis through modification of SH-groups of GAPDH [266]. NO increases during stress and consequently reduces performance by inhibiting ATP production. Attenuation of NO production can be attained by inhibition of i-NOS activity and by inhibition of the synthesis of this enzyme at the transcriptional level which can be achieved by inhibition of mitogen-activated protein factor JNK as it is known to activate expression of i-NOS [270]. It has been shown that extracts of *E. senticosus* inhibit lipopolysaccharide (LPS) and interferon-induced NO production [271] and attenuate the expression of both i-NOS and i-NOS m-RNA in through the inhibition of JNK pathways [260]. Hence another mechanism which may be postulated for drugs with antistress effect is...
that they might prevent the stress-induced increase in NO and, consequently maintain efficient ATP production and thus causing increased performance and endurance [267,272]. It has been also demonstrated that some combinations of herbs with antistress effects e.g ADAPT-232 increases the expression of GAPDH by 2-fold in the hippocampus of rats [273]. The beneficial effect of antistress preparations like ADAPT-232 is probably related to the up regulation of Hsp 72, which in turn stimulates the immune system and reverses the process of protein denaturation which is dramatically increased in the acute phase of inflammation [274]. It is a known fact that adaptogens might act by enhancing the capacity of the body to respond to stress stimuli by activating/deactivating mediators of response to stress, as corticosteroids, catecholamines and nitric oxide [267,26,275]. Among the several activities mentioned, the modulation of the hypothalamic-pituitary-adrenal axis (HPA) is probably the most studied, and seems to be one of the main target of drugs with antistress effect [233,235]. Under situations of chronic stress, drugs with antistress effect would act by reestablishing the functioning of the axis, stopping the liberation of stress hormone by reestablishing the sensitivity of receptors involved in the negative feedback mechanism. The role of drugs with antistress effect in the HPA would take place mainly by promoting a positive regulation of certain stress modulators, especially the heat shock protein HSP70, which plays a key role in the apoptosis and cell lifespan [276]. .Another system that seems to play an important role in the set of positive actions of drugs with antistress effect is the immune system. Drugs with antistress effect may also act in a nonspecific way, through its immunomodulating activities [253]. A number of plants considered to exhibit antistress effect are immunostimulants e.g. Eleutherococcus senticosus, Withania somnifera, Bryonia alba, Ocimum sanctum L. Lamiaceae, and the Panax ginseng, as well [233,235,47,277-279]. Evidence shows that the immune, sympathoadrenal systems and the HPA axis (stress system) share several mediators with effects on target organs in common [280,281]. The adaptogen might show beneficial effect by increasing the mobilization of energy sources and preventing abrupt reaction to stress [34,279].Itis also reported that antioxidant property certainly contributes to the set of actions of drugs with antistress effect. [282,283] which reinforces the importance of this activity by the plants to be used to decrease the deficits resulting from aging. Some examples are Ginkgo biloba [284], Schisandra chinensis [285], Rhodiola rosea [286] and Eleutherococcus senticosus [287]. Since drugs possessing antistress effect act by improving cognitive performance, it is believed that adaptogens can also modulate the cholinergic system and other neurotransmission systems like monoaminergic neurotransmission [288,289]. Other targets that seem to contribute to the action of drugs
exhibiting antistress effect include the modulation of genic transcription and protein synthesis, and the modulation of the several glycolysis phases as discussed earlier. Thus it can be concluded that under stress drugs with antistress effect help the adrenal glands to mount an immediate hormonal response, by manufacturing and releasing more stress hormones whereas on termination of stress, they help the adrenal glands to shut down more quickly. If stress is prolonged and severe they help to restore hypothalamic receptor sensitivity thereby, the adrenal glands reserve their resources by reducing the amount of hormones released. This conserved energy is available to continue the body’s responses to stressors, thereby delaying adrenal exhaustion. Adaptogens lower plasma corticosterone levels, thus reducing the damage caused by excess cortisol. In other situations they potentiate cortisol and ACTH, sparing the breakdown of cortisol delaying the exhaustive state. Being able to modulate either deficient or excess conditions is characteristic of a true adaptogen. The biological machinery that handles energy in the body is stimulated by drugs with antistress effect. Consequently, during stress, more glucose is released into the blood from the body’s storehouses. Adaptogens help glucose to cross the cellular membranes more easily. As a result glucose is quickly taken up by the tissues to carry out their work. Hence preventing stress induced hyperglycemia and increasing tissue work efficiency. Thus drugs exhibiting antistress effect assist the body because of their ability to normalize homeostasis, optimize metabolism, revitalize exhausted organ systems, and improve resistance to a variety of adverse factors without side effects. Certain drugs with antistress effect have a profound anabolic effect as they help the body to rebuild damaged muscle tissue rapidly. They improve sleep and cognition that might otherwise be disrupted during stress. Some adaptogens involve immune-modulation as well. Drugs possessing antistress activity balance and rejuvenate the yin (inward) and yang (outward) energy. They help us to cope with stress more effectively, psychologically, mentally and emotionally. They also resist or delay many of the negative effects of aging by providing us with better physical, mental and sexual energy. Therefore, herbal drugs with antistress effect hold great promise for the development and prevention of chronic illness due to their ability to enhance our resistance to a variety of stressors nonspecifically.
<table>
<thead>
<tr>
<th>Sr. No.</th>
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<th>Botanical name</th>
<th>Pharmacological effects</th>
<th>Ref.</th>
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<td>1.</td>
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<td>[290]</td>
</tr>
<tr>
<td>2.</td>
<td>American Ginseng root</td>
<td>Panax quinquefolius</td>
<td>Adaptogen, antioxidant, antiinflammatory, bitter tonic, immune amphoteric</td>
<td>[291]</td>
</tr>
<tr>
<td>3.</td>
<td>Eleuthero root</td>
<td>Eleutherococcus senticosis</td>
<td>Adaptogen, anticholesteremic, antioxidant, antiinflammatory (mild), immune potentiator, nervine.</td>
<td>[47]</td>
</tr>
<tr>
<td>4.</td>
<td>Wu Wei Zi berries/seeds</td>
<td>Schisandra chinensis, S. splenathera</td>
<td>Adaptogen, antioxidant, antiinflammatory, astringent, antiasthmatic, hepatoprotective, immune amphoteric</td>
<td>[229]</td>
</tr>
<tr>
<td>5.</td>
<td>Ashwagandha root</td>
<td>Withania somnifera</td>
<td>Adaptogen, antiinflammatory, antioxidant, antispasmodic, astringent, immune amphoteric, sedative (mild)</td>
<td>[37, 38]</td>
</tr>
<tr>
<td>6.</td>
<td>Shatavari roots</td>
<td>Asparagus Racemosus</td>
<td>Adaptogen, anti oxytocic, antiepileptic, antileptoropic, antiulcer, antiinflammatory, antipyr etic, analgesic</td>
<td>[293]</td>
</tr>
<tr>
<td>7.</td>
<td>Dang Shen root</td>
<td>Codonopsis pilosula</td>
<td>Adaptogen, gastroprotective, hypoglycemic agent, immune potentiator, nervine</td>
<td>[294]</td>
</tr>
<tr>
<td>8.</td>
<td>Licorice rhizome</td>
<td>(Glycyrrhiza glabra, G. uralensis)</td>
<td>Adaptogen, antihistamine, antiinflammatory, antidiuretic, antioxidant, antitussive, antiviral, demulcent, hepatoprotective, immune amphoteric, gastroprotective</td>
<td>[295]</td>
</tr>
<tr>
<td>9.</td>
<td>Cordyceps fungus</td>
<td>Cordyceps sinensis</td>
<td>Adaptogen, antiasthmatic, antileukemic, antioxidant, hepatoprotective, immune potentiator, nephroprotective, sedative (mild)</td>
<td>[296]</td>
</tr>
<tr>
<td>10</td>
<td>Holy Basil herb (Tulsi):</td>
<td>Ocimum sanctum</td>
<td>Adaptogen, antibacterial, anticholesteremic, antidepressant, antioxidant, antiviral, carminative, expectorant, immune amphoteric.</td>
<td>[39]</td>
</tr>
<tr>
<td>11</td>
<td>Rhodiola root</td>
<td>Rhodiola rosea, R. crenulata</td>
<td>Adaptogen, antiinflammatory, antioxidant, antidepressant, cardioprotective, immune potentiator, nervine.</td>
<td>[297, 298]</td>
</tr>
<tr>
<td>12</td>
<td>Amla fruit</td>
<td>Emblica officinalis</td>
<td>Adaptogen, antioxidant, anticholesteremic, antiinflammatory, astringent, radioprotective, thyroxin inhibitor, diuretic, hepatoprotective (mild), nutritive</td>
<td>[299]</td>
</tr>
<tr>
<td>13</td>
<td>Bryonia root</td>
<td>Bryonia alba</td>
<td>Adaptogen, antiinflammatory, analgesic antibacterial, antioxidant, cardiotonic, immune amphoteric</td>
<td>[235]</td>
</tr>
<tr>
<td>14</td>
<td>Jiaogulan herb</td>
<td>Gynostemma pentaphylla</td>
<td>Adaptogen, antioxidant, expectorant, hypocholesteremic, hepatoprotective, immune potentiator, nervine.</td>
<td>[300]</td>
</tr>
<tr>
<td>15</td>
<td>Guduchi stem</td>
<td>Tinospora cordifolia</td>
<td>Adaptogen, antiinflammatory, antioxidant, hepatoprotective, diuretic, immune amphoteric.</td>
<td>[26]</td>
</tr>
</tbody>
</table>

**Table 2.2.4**

Medicinal plants reported in literature with antistress effect (adaptogens)
2.2.6. Review of literature of some medicinal plants reported with antistress effect:

a) *Panax ginseng* (PG)

The tonic effect of the ginseng root has been described in a Chinese text as early as the 1st century after Christ. According to current understanding, the adaptogenic effect of the drug is ascribed to the ginsenosides or panaxosides diversely glycosylized triterpene saponins which, with the exception of ginsenoside R0, belong to the tetracyclic dammarane-type. Ginsenoside R0 has oleanolic acid as the aglycone. The chief glycones are the ginsenosides Rb1 and Rg1. Additional constituents include essential oil, the sesquiterpene beta-elemene, polyacetylenes, salicylic- and vanillic-acid polysaccharides as well as ubiquitously occurring amino acids, fatty acids, sterines and sugars [301].

*P. ginseng* has been researched since the 1960s in order to understand how it exerts its nonspecific action. Extrapolations from pharmacological studies have revealed that *P. ginseng* has a profound effect on the whole body as it aids in the resistance to stress and aging. During stress the adrenal glands secrete catecholamines and cortisol from the medulla and the cortex which can lead to disease if left unchecked for too long. The effects of Korean ginseng isolates on functions of adrenal medulla were investigated in vitro and it was found that the saponin rich fraction greatly reduced the secretion of catecholamines whereas the non-saponin fraction did not affect it at all [290].

A comparative study of *Panax ginseng* and *Ginkgo biloba* for assessing the antistress effects was performed by Rai et al (2003). In this study rats were subjected to acute stress and chronic stress after the treatment with the herbs. The effect on various biochemical parameters and on occurrence of gastric ulcer were examined. The researchers found that *Ginkgo biloba* is more effective in acute stress whereas *Panax ginseng* is a better option for chronic stress [304]. According to the literature reviewed the mechanisms of action that are responsible for this effect are those that resist stress via an antioxidant action, those that act on the CNS and those that regulate neuro-endocrine level pathways [303]. Sha et al (2005), Kim et al (2005) and Oliveira et al (2005) in four different studies observed that *P. ginseng* exhibit a strong antioxidant action. The prevention of myocardial damage from post ischemic reperfusion occurred as *P. ginseng* quenched oxygen radicals, saving the heart endothelium from oxidative stress. Additionally it was proposed to stimulate the transcription of cupric zinc superoxide dismutase (Cu/Zn SOD) or coronary endothelium nitric oxide (NO) synthase.
and endothelial cyclic GMP production of NO which is now considered to be an important antioxidant (Facino 1999). By testing *P. ginseng* on a focal model of ischemia, Shah et al have attributed the herb's protective effect on ischemic-induced neuronal damage to an antioxidant action where lipid peroxidation was found to be reduced and levels of various scavenger enzymes were increased. Lipid peroxidation (LPO) is known to occur during stress as a nonspecific response and scavenger enzymes are believed to enhance defence of neuronal tissue leading to a reduction in damage [304]. Kim et al also found that *P. ginseng* exerted an antioxidant action in a study to test its effect on exercise induced exhaustion. It was reported to have stimulated the production of scavenger enzymes leading to a decrease in lipid peroxidation [305]. De Oliveira et al found *P. ginseng* to be protective for muscle during exercise due to a reduction in protein oxidation [306]. Another indicator of the adaptive response to exercise is an increase in capillary density and mitochondrial volume. Ferrando et al (1999) found that *P. ginseng* exerted a similar effect to exercise on muscle oxidative capacity and capillary density however it did not have a cumulative effect when administered during exercise [307]. The adaptogenic effect of *P. ginseng* is also believed to occur due to its action on the HPA axis of the neuroendocrine pathway. According to the review by Nocerino et al adaptation is enhanced due to ginsenoside stimulation of steroidogenesis by indirectly acting on the pituitary gland. In this way it is believed to affect memory, learning and exercise endurance [305]. Their results showed decreases in adrenaline and prolactin (in older rats) and increases in adrenocorticotrophic hormone (ACTH) in both young and old rats with *P. ginseng* administration. Because ACTH and adrenaline are involved in the neuroendocrine stress response these studies suggested that up or down regulation of these hormones may affect the adaptive response. Other CNS mechanisms were observed by Provalova et al, Park et al (2002). A reduction in bone marrow erythropoiesis during paradoxical sleep deprivation (PSD) was used as a study model to test the effect of adaptogens. *P. ginseng* in this study, was seen to stimulate bone marrow erythropoiesis during PSD via modulation of neurotransmitter systems including serotonergic and cholinergic structures in the brain [308]. Another study testing the neuroprotective effect of *P. ginseng* by Park et al (2005) on a hypoxic ischemic model revealed a protective effect that could be attributed to an increase in calmodulin-dependant kinase II (CaMKII) [311]. Ni et al (1993) tested the effect of *P. ginseng* using a spatial working memory disruption model. *P. ginseng* was seen to dose dependently improve maze performance disruption and performance deficits. Interaction of *P. ginseng* with cholinergic function was suggested as the main mechanism of action [310].
**b) Eleutherococcus senticosus (ES)**

In search of a drug which could replace the expensive ginseng root, one came across the Taiga root, originating from Siberia. The chief constituents are considerably different than those of the ginseng root which includes [311]. Phenyl propane compounds like syringin eleutheroside B, sinapin alcohol, coniferyl aldehyde, chlorogenic acid, caffeic acid derivatives. Lignanes: syringaresinol-4-4'-0-beta-D-diglucoside = Eleutheroside E (D), syringa-resinol monoglucoside, syringaresinol, sesamin. Coumarins: e.g. Isofraxidin-7-0-glucoside and its aglycon, isoferaxidin., Polysaccharides.and additional constituents, such as sterins, oleanolic acid, essential oil, sugar [312]. E. senicocus showed activity on the HPA axis and on enzyme inhibition; P. ginseng demonstrated activity on the CNS ,on the HPA axis, on mitochondrial function, and also demonstrated antioxidant action; In a study by Kimura et al (2004) swimming time, natural killer (NK) activity and corticosterone levels in forced swimming stressed mice were reduced by E. senticosus root bark in which eletheroside E was found to be present [279].

As it had been reported that glucocorticoids play a role in adaptation to stress via the neuroendocrine pathway and immune systems (Panossian 1999a), these results supported the theory that adaptogens may modify stress via an effect on the HPA axis and the sympatho-adrenal system (SAS). Another study testing the effect of E. senticosus on mast cell dependent anaphylaxis demonstrated an inhibitory effect on IgE-induced production from mast cells and inhibition of mast cell mediated anaphylaxis. Although the results of this study were promising , the dose in this study was too high to be of therapeutic significance. However speculation that E. senticosus may regulate mast cell granulation by stabilising membrane fluidity was postulated [313].

E.Senticocosus was found to be significantly reducing adrenal hypertrophy and adrenal ascorbic acid depletion and demonstrated sparing effect on the adrenal cortex .these observations indicated the property of plant to impart an ability to the organism to better withstand with prolonged stress. Siberian ginseng can act in different ways to support the body during times of stress and this is dependent on what stage the stress response is at. Research suggests that there is a threshold of stress below which the herb increases the stress response and above which it decreases the stress response, and various mechanisms have been proposed as to how ES works including inhibition of catehol-O-methyl transferase, an enzyme which inactivates catecholamines . As a result catecholamine levels are not depleted and release of new catecholamines from nerve synpases is decreased. Eleutherosides have
also been shown to improve carbohydrate metabolism and energy provision and increase the synthesis of protein and nucleic acids thus theoretically might be helping to prevent the exhaustion stage of the stress response. But this is just a theory and more concrete evidence is required. As part of its adaptogenic effect Siberian ginseng may also exert neuroprotective, hepatoprotective and cardioprotective activity. Hence it is an example of a herb with holistic characteristics as an adaptogen that also helps to protect vital organs [314]. Numerous studies have been carried out by using Siberian ginseng in combination with other adaptogens, such as *Rhodiola rosea* and *Schisandra chinensis*, showing potentially synergistic action for improved antistress effects.

c) *Rhodiola rosea* (RR)

*Rhodiola rosea* is used by the ancient Siberians for the prevention of tiredness and reduced interest in work [315]. Besides salidrosid, the thyrosol-glucoside, cinnamol alcohol glycosides are considered to be the active constituents. Especially notable is the rosavidin, the cinnamyly-0-(6’-0-L-arabinopyranosyl-D-glucopyranosid) Additional constituents are thyrosol and cinnamic alcohol, essential oil, anthraglycosides, beta-sitosterin, daucosterol, monoterpenes, flavonoids and 16-18% tannins [316].

Research into the adaptogenic effects of the herb has revealed that there are many different species of Rhodiola, however R. rosea is the most extensively researched. Hypotheses from scientific research as to how learning and memory is affected by R. rosea include activity on neurotransmitters in neuronal pathways, suppressed inhibition of acetylcholine (ACh) with age associated memory loss and reduction in oxidative damage all of which are consequences of stress. Original research by Hillhouse et al(2004) supported ACh modulation as an acetylcholine esterase (AChE) inhibitor which may be the mechanism of action for improved memory[317]. R. rosea had been found to reduce symptoms of physically and psychiatrically induced asthenia and to increase intellectual capacity. It has been shown to improve the effects of tricyclic antidepressants (TCA) and decrease their side effects. Dopaminergic activity has been suggested as a mechanism behind relieving parkinsonian symptoms as a side effect of neuroleptics [318]. Other reviewed studies suggested that increased resistance to nonspecific stress by herb may be due to a serotonergic action, an increase in [beta]-endorphins and moderated level of opioid peptide, an excess of which may damage the brain and heart. R. rosea was found to act on the neuroendocrine system similarly to other adaptogens and to have strong antioxidant properties where toxicity from drugs is decreased
Review of Literature

and some anticancer drug actions can be enhanced [318]. Ohsugi et al (1999) isolated 19 active compounds which had oxygen scavenging activities against superoxide anion radical and hydroxyl radical. They have hypothesised that the antiaging activity attributed to the plant may be due to oxygen scavenging that reduce imbalanced redox reactions and restore defence against free radicals [319]. In a study investigating the antioxidant potential of three adaptogen extracts, Rhodiola rosea, Eleutherococcus senticosus and Emblica officinalis it was found that rhodiola had the highest potential for singlet oxygen scavenging, hydrogen peroxide scavenging, ferric reducing, ferrous chelating and protein thiol protection over either of the other two extracts. The highest polyphenol content of Rhodiola was thought to be responsible not only for observed adaptogen properties but also for decreasing the risk of complications induced by oxidative stress[250]. Induction of iNOS gene expression by R. sachalinensis leading to NO synthesis was another proposed mechanism of action [320]. As changes in ATP content in mitochondria are indicative of stress from exercise. Abidov et al (2004) suggested that improved tolerance to exercise in an exhaustive swimming test model was may be due to stimulation of ATP production [321]. While Boon-niermeijer et al (2000) found R. rosea to be protective against lethal heat shock and superoxide radical [322]. The hypothesis that synthesis of stress proteins as an adaptive response responsible for this action was excluded. Additionally a study to test the effect of Astragalus and Rhodiola species on noise stress revealed reduction in hepatic glycogen, lactic acid and cholesterol which may be ultimately controlled by the HPA axis as an adaptive response [323]. An anti-inflammatory action was seen as a mediator of adaptation as levels of C-reactive protein (CRP) and creatine kinase (CK) were reduced in untrained volunteers before exercise in treatment group [264]. In another study using rabbits, the objective of which was to ascertain which mediators of stress response are significantly involved in the mechanisms of action of adaptogens and to determine their relevance as biochemical markers for evaluating antistress effects. It was suggested that the inhibitory effects of R. rosea and Schisandra chinensis on phosphorylated kinase p-SAPK/p-JNK activation may be associated with their antidepressant activity as well as their positive effects on mental performance under stress [267].

d) Schisandra chinensis (SC)

Schisandra chinensis is another herb viewed as an adaptogen, particularly in Traditional Chinese Medicine. Schizandrin is one of the main dibenzocyclo octadiene lignans present in the fruit of Schisandra chinensis. Biological activities including hepatoprotective, antiviral
and neuroprotective effects of schizandrin and other dibenzocyclooctadiene lignans have been reported.[324]. Recent studies have demonstrated that schizandrin exhibits antioxidative effects in mice [325]. Other chemical constituents include γ-terpinene, bisabolene (+)-gomisin K2, gomisin S, pregomisin, schisantherin A, schicantherin B, angeloylgomisin Q, and rubrildilactione [328].

According to a review by Hancke et al(1999) S.C was found to exert its antioxidant effect by increasing GST levels in liver and decreasing concentration of lipid per oxidation by product Malondaldehyde (MDA).form these observation antioxidant property of the herb was thought as may be largely responsible for its adaptogenic activity [327] Ip et al (1995), Zhu et al (1999) and Chiu et al (2002) observed modulation of the hepatic detoxification enzymes including of GST, glutathione reductase (GRD), 6-phosphate dehydrogenase and [gamma]-glutamylcysteine, Cyt P450, serum glutamic oxaloacetic transaminase (SGPT) and serum glutamic pyruvic transaminase (SGOT) and these have been associated with hepatoprotection from CCl4. Toxicity [262,263,328] Its effect on physical performance had been associated with reductions in transaminase, creatine phosphse kinase (CPK) and lactate levels as well as to increases in antioxidant status as measured by decreases in LPO and MDA. An observed anticarcinogenic effect of the herb may be due to a stimulating effect on cytochrome P450 enzyme action and GST which are associated with the elimination of polycyclic aromatic hydrocarbons (known carcinogens). [328] isolated lignins of S. chinensis were found to reduce in intracellular calcium thereby causing protection of glutamate induced toxicity. NO is driven by calcium dependant channels and is also associated with glutamate toxicity due to the generation of reactive oxygen species (ROS) by NO. A decrease of NO due to a reduction in calcium and therefore calcium dependant enzymes was the proposed mechanism of action. Panossian et al (1999) also demonstrated a connection between S. chinensis and NO. Salivary NO was seen to increase after treatment to athletes and was found to decrease after physical exercise in same atheletes. In the same study a rise in cortisol on treatment and a decrease after physical exercise were also observed. Because NO and cortisol play a role in the switch on (NO) switch off (cortisol) phases of the neuroendocrine stress axis they may be used as markers for stress and their activation and suppression may be indicative of the adaptive process. The authors concluded from this that S. chinensis has a pro-stressor effect as NO and cortisol are stimulated in order to improve adaptation [229]. A study by Hernandez et al (1988) on the other hand claimed that S. chinensis had no effect on stress induced gastric ulceration which
suggested that the cortisol-immune pathway may not have been affected by herb [329]. Hancke in his review of S. chinensis, discussed the possibility of CNS stimulant effect of S.C as barbiturate activity was found to be inhibited under experimental conditions [285].

**e) Withania somnifera (WS)**

*Withania somnifera* has immunomodulatory, anti-inflammatory but most significantly adaptogenic effects, which may result from the complex of the many steroidal withanolides found in the root of the herb (Mills 2000, Braun 2007). The root of the plant is considered as a tonic. It is found to "protect the organism from illness through maintaining the balance of the physical energies [37]. The root contains the steroid lactone withaferin A and related withanolides, beside various alkaloids. The sitoindosides IX and X isolated by Ghosal et al. represent C-27-glycowlanolides [38] the sitoindosides VII and VIII, acylesterylglucosides [37]. Kaur et al tested the adaptogenic activity of W. somnifera by administering a previously untested withanolide compound (1-oxo-5[beta], 6[beta]-epoxy-witha-2-ene-27-ethoxy-olide) on rats in a cold-hypoxia-restraint model (C-H-R-Stress) [330]. Stress parameters were reduced in treatment group compared to controls and blood parameters revealed a decrease in CPK, lactase dehydrogenase (LDH) and LPO in treatment group compared to controls. There was also a reduction in serum corticosterone in treatment group compared to controls. While the authors did not specifically refer to antioxidant action as such, they concluded that an increased tolerance to stress was in part due to a decrease in CPK, LDH and LPO. Reduction in corticosterone levels during stress suggested HPA axis activity. W. somnifera was also observed to suppress OVA-specific IgE antibody and to down regulate OVA-specific IgE antibody response [331]. The IgE hypersensitivity response is an example of poor adaptation to stress. In testing adaptogenic and cardioprotective actions as well as biochemical parameters of blood coagulation in the forced swimming test model, upregulation of anabolic processes and activity on catecholamine and mitochondrial processes was postulated as a possible mechanism of action [332]. Battacharya et al (2002) attributed the mechanism of action to an antioxidant effect rather than CNS activity causing reduced symptoms in a tardive dyskinesia model [333]. In another study swimming time was found to be increased (p<0.01) and body weights of mice were also increased (p<0.05) significantly in treatment groups compared to controls groups subjected to swimming stress model. The anabolic activity of the herb was attributed to the presence of steroid compounds. W. somnifera contains steroidal lactones called withanolides. It was also postulated that the observed anabolic effect may be due to an anti-seratonergic activity which would lead to an
increase in appetite and therefore weight gain [334]. In another paper Battacharya et al (2000) attributed the anxiolytic and antidepressant actions of isolated glycowithanolides to GABA-mimetic activity [335]. Activity on the HPA axis was postulated as a mechanism of action by Battacharya et al (2003) in a study to test the adaptogenic effect of W. somnifera on chronic stress using a foot shock model. The symptoms of stress from footshock manifest in a variety of nonspecific maladies including gastric ulcer, hyperglycemia, glucose intolerance, increased plasma cortisone, sexual dysfunction in males, cognitive deficits, immunosuppression and mental depression. For all of the above symptoms administration of W. somnifera was found to decrease the degree of stress induced by foot shock. However, this paper did not specify exactly how W. somnifera acts upon the HPA axis and increases the resistance phase of the stress response in order to prevent exhaustion. This particular test supports its reputation as having a wide ranging action which is non-specific [336]. In one another study conducted on rats investigated the effect of WS root extract on sleep and its possible interaction with GABAergic modulators on the sleep wake cycle. In this study pretreatment with WS shortened sleep latency, decreased waking, increased non rapid eye movement and total sleep time [337]. As stress also induces insomnia so this observed effect may be considered as one of the role that withania somniferii plays in stress management. Chronic stress depresses immune functioning and increases susceptibility to disease. A recent study was conducted on Swiss albino mice to see the effect of withanolide A isolated from WS root extract on chronic stress induced alterations on T lymphocyte subset distribution and corresponding cytokine secretion patterns. Treatment with Withanolide A caused significant recovery of stress induced depleted T cell population causing an increase in the expression of IL-2 and IFN-gamma (a signature cytokine of Th1 helper cells) and a decrease in the concentration of corticosterone. This study supports role of WS in stress management including immune function [338]. Impairment in memory in one of the consequences of chronic stress. In another study researchers investigated the effect of withanolide A on memory deficient mice showing neuronal atrophy and synaptic loss in the brain. Treatment with withanolide A induced significant regeneration of both axons and dendrites, in addition to the reconstruction of pre and postsynapses in the neurons [339].
2.3. Review of literature of *Murraya Koenigii* (Curry leaf) and *Ocimum Sanctum* (Krishna Tulsi).

2.3.1. *Murraya Koenigii*

Curry leaf, (*Murraya Koenigii* (L)) is an aromatic shrub found almost throughout India up to an altitude of 1500 meter. Plant is found commonly distributed in kokan western ghats of Bombay to Travancore and Ceylon and in most of the districts of Madras and along the foot of Himalaya and Bashahi eastwards to sikkim and Peninsular [340]. Commercial cultivation in India is limited to Tamilnadu and karnataka states. It is much cultivated for its aromatic leaves as they are extensively used as flavouring agents in curries and chutney [52,341]. The plant live up to 30 yrs and go on producing aromatic leaves throughout the year. The flowering of the plant starts from middle of April and ends in middle of May. The fruiting season is observed to be continuing from middle of July to the end of August [342]. The root bark and leaves are bitter, acrid, astringent and cooling in taste. The whole plant is considered as tonic stomachin and carminative. Especially leaves are used to promote appetite, digestion and during dysentery, diarrhoea and to stop vomiting and to destroy pathogenic micro-organisms [52]. It is reported to be useful in emaciation, skin diseases, worm troubles, necrosis and poisons. Roots are antiprotozoal and its juice is used to relieve pain associated with kidney [340]. The stems are very popular for cleaning teeth and are said to strengthen the gums and teeth. [343]. Essential oil obtained from leaves is useful in soap industry and perfume industry [344]. *M koenigii* is known to be the richest source of carbazole alkaloids which are reported to possess various biological activities such as antitumor, antioxidative, antimutagenic and antiinflammatory [345,346].

**Morphological Characters:**

*Murraya Koenigii* is unarmed small tree up to 6 cm in height and 15-40 cm in a diameter with dark gray bark and closely crowded spreading dark green foliage (52). Leaves are imparipinnate, alternate and compound, about 30 cm long. Each bearing 11-25 alternate leaflets (2.5 by 1.25 cm) which are obliquely ovate, acuminate, obtuse or acute and gland dotted [347]. The girth of the main stem is 16 cm. Flowers are bisexual, white, funnel shaped, sweetly scented stalked and arranged in much branched terminal corymbose cymes. Each flower is actinomorphic, pentamerous, hypogonous, with diameter of fully opened flower.
being 1.12 cm. Inflorescence, a terminal cyme each bearing 60 to 90 flowers, five lobed calyx, inferior and green corolla, white polypetalous with 5 petals, lanceolate with petal length 5 mm [340]. Fruits are subglobose or ellipsoid berries. They are pulpy, purplish black with shining surface when ripe and two seeded. Each fruit is 1.4-1.6 cm long, 1 to 1.2 cm in diameter. The number of fruits per cluster varies from 32 to 80 [.348].

**Synonyms**

Sanskrit: Kalasakh, Kaidaryah:
Marathi: Kadhipatta
Hindi: Mithinim, Katnim
Malyali: Kariveppa, Karuveppu
Tamil: Karivepilaai, Karuveppu
Kannada: Karibueva
Telugu: Karivepaku
Assameese: Narasingha, Bishahari
Bengali: Barsunga
Gujarati: Goranimb, Kadhilimbdo

_Murraya Koenigii_
Phytochemistry

I). Leaves:

1 Alkaloids in Leaves.

Kureel et al (1969) isolated number of alkaloids including cyclomahanimbine bicyclomahanimbine mahanimbidine koenimbine & koenigicine from leaves of *Murraya Koenigii* [349]. Four more alkaloids were then isolated by Narsihan et al (1970) isolated from acetone extract of leaves were mahanine, koenine, koenigine and koenidine showing the presence of angularly fused pyranocarbazole skeleton.[350]. In the hexane extract of the plant along with mahanimbine and girinimbine the presence of two new alkaloids isomahanimbine and koenimbidine had been reported [351]. Leaves were also found to yield mahanimbinine and [352] Mahanimbidine [353] .Among these alkaloids the chemical structures were determined by synthetic studies for koenine, Koenigine, mahanine & isomahanimbine [354] mahanine [355]. Koenigiline [356]. One more new carbazole alkaloid mukonicine was obtained from alcoholic extract of defatted leaves of *Murraya Koenigii* by Mukherjee et al (1983) [357]. Chakroborti et al (2009) reported presence of another new cabazole alkaloid designated as murrayakoeninol in the leaves of *Murraya koenigii* [358]. Coumarine glycosides isolated from leaves are scopoline and crystalline glucoside isolated is koenigine [359]. A new binary carbazole alkaloid, 8, 8 α-biskoengine along with its monomer, koenigine, was isolated from the dried leaves of *Murraya koenigii* [360,361].

2. Chemical structures:

![Mahanimbine](image1)

![Girinimbine](image2)

![Isomahanimbine](image3)

Mahanimbine  
Girinimbine  
Isomahanimbine
Review of Literature

Mahanine  
Koenimbine  
Koenigine

7.

Koenidine  
Koenine  
Murrayazolidine

9.

Murrayafoline  
Murroyazoline  
Murrayazolidine

Mahanimbidine  
Cyclomahanimbidine
3. Essential oil in leaves:

The fresh curry leaves contain 2.6% volatile essential oils (containing sesquiterpenes and monoterpenes [362] which are sufficiently soluble in water. [363]. Majority of the chemical constituents detected in the leaf oil of Murraya koenigii were mainly sesquiterpene hydrocarbons. Dutt et al (1958) reported that the essential oil obtained by steam distillation under pressure from fresh leaves of *M. Koenigii* yielded dl-2 phellandrene, d-sabinene, d-α-pinene, dipentene d-α-terpinol, isosafrol caryophyllene cadinene, cadvirol, lauric acid & palmitic acid [364]. Nigam et al (1961) found that the major constituents responsible for aroma & flavour of the oil include 1-α-pinene, 1-sabinene dipentene, 1-terpinol-1-caryophyllene and 1-cadinene [365]. Chowdhary et al (1999) reported that leaves on hydrodistillation yield 0.5% essential oil on fresh weight basis, having dark yellow colour, spicy odour and pungent clove-like taste [366]. In year 2008 chemical composition of essential oil from the leaves of *Murraya Koenigii* was studied by GCMS and was found to contain thirty nine compounds of which a major is 3-carene (54.22%) followed by caryophyllene (9.49%) other notable compounds. were α-thujene (1.47%) allyl (methoxy) dimethylsilane (2.58%), β-myrcene (3.2%) α-terpinene (2.39%), γ-terpinene (2.7%) cis-subinene hydrate (1.467) 4-terpineol (2.8%) β-elemene (1.92%) α-caryophyllene (2.81%) relemene (1.96%) caryophyllene oxide (1.02%) and 3-phenyl butyrophenone (1.15%) [367]

4. Nutrients in leaves

Leaves of *Murraya koenigii* possess high nutritive value. as observations on analysis revealed the presence of protein (6%) fat (1%) carbohydrates (16%) fiber (6.4%) minerals (4.2%) which includes calcium (810mg), phosphorus (600mg) iron (3mg) and also vitamins such as carotene (Vitamin A) (12600IU), Nicotinic acid (2.3mg), Vitamin C (4mg / 100g). Amino acids present include asparagin, glycine, serine, aspartic acid, glutamic acid, theonine, alanine, proline, tyrosine, Tryptophan, γ amino buturic acid, phenyl alanine, leucine, isoleucine, lysine, Arginine & histidine. It also contain 0.8% potash [344]. In studies carried by Usha et al (2002) on finding out an ideal temperature for storage of curry leaves, the highest concentration of lutein was found in fresh leaves, α-tocopherol concentrations of the fresh leaves and air dried leaves were found to be unaffected by freezing, whereas oven-drying lead to 50% loss of α-tocopherol. The β-carotene concentration was observed to be highest in fresh leaves and lowest in the oven-dried leaves. The fresh and the frozen curry
leaves had similar chlorophyll concentrations Oven-drying resulted in a greater loss (~64%) of chlorophyll compared to air-drying (~32%)[368].

II. Stem & bark:

Pet ether extraction of stem bark of Murraya koenigii gave a number of Carbazole alkaloids which includes girinimbine [369], Murrayanine [370,371], mahanimbine, Murrayacine [372], Mukanol [373]. Murrayazolidine [374]. The other alkaloid murrayacine isolated was an oxidative variant of mahanimbine.[375] (40). Some more alkaloids reported from stem bark include Isomurrayazoline [376] curryanin curryangin [377] Murrayazoline [378]. Eventually curryangin murrayazoline from stem bark & mahanimbidine [379] were reported to share same chemical structure. And also murrayazolidine from stem bark & cyclomahanimbine from leaves were reported to have similar structure [380]. Alcoholic extract of stem of, Murraya Koenigii yielded mukoeic acid [381].(46) & methyl ester of mukoeic acid, Mukonine was isolated from petrol extract of stem bark[382].

III. Fruits:

The fruit is edible. & imparts pepper like taste which imparts sensation of coolness on the tongue [369]. The fruit yields 0.76% of a yellow, volatile oil with a peppery taste, and also contains the glucoside, koenigin [383]. (48) (50) Pulp of fruit contains sugar (9.26%) Vitamin C(13.35 mg / 100 g) & protein(1.97mg / 100g) phosphorus, potassium calcium, magnesium & iron [342].

IV. Seeds:

A yellow clear and transparent oil is procured from the seeds which is known as limbolee oil. It yields 0.76 % of yellow volatile oil with Neroli like adour Furcoumarins reported to be isolated from seeds of Murraya Koenigii are Gasferol, byakangelicol, Isogasoferol, byakangelicin [384]. Two new carbazole alkaloids, isomahanine and murrayanol were isolated, from the seeds along with five known carbazoles, mahanimbine, murrayazolidine, girinimbine, koenimbine and mahanine [383].

V. Roots:

Light petroleum extract of roots of Murraya Koenigii yielded mahanimboline[385]. The hexane extract yielded girinimbine [351]. The benzene extract yielded mukoline & mukolidine [386]. Air-dried roots of Murraya koenigii when defatted with petrol and then
extracted with CHCl3 , the compound 3-formyl 1,8-dimethoxycarbazole, was isolated.[387] Two carbazole alkaloids 3-methylcarbazole and murrayafoline A were isolated from the petroleum ether extract of the roots of Murraya koenigii for the first time from this plant [388] amongst which murrayafoline A was reported previously as low melting solid isolated from Murraya euchrestifolia [389].

**Pharmacological actions:**

1. **Hypolipidemic activity:**

*Murraya Koenigii* which is a very common constituent of Indian food is screened by number of scientists for its hypolipidemic potential some of those studies are oriented to explore its role in controlling hyperlipidemia especially the one which is commonly observed in diabetic patients while the other studies involve evaluation of it’s hypolipidemic effect by including the plant in daily diet. It is observed that on chronic administration of curry leaf along with diet containing 10% coconut oil to rats for 90 days resulted reduction in total serum cholesterol, LDL, VLDL levels , decreased release of liporotein , increased lecithin cholesterol acyl transferase and also increased HDL levels in blood [390]. Where as in another study aimed at observing the effect of curry leaves supplementation for comparatively smaller period of 15 days in non insulin diabetic patients., no appreciable changes in glycosylated low density lipoprotein , cholesterol fraction , serum lipids, lipoprotein, cholesterol levels were observed but at the same time there was transient reduction in fasting and postprandial blood sugar level[391]. In another study curry leaf powder was administered in beef sausage and effect on blood cholesterol level in mice was observed. In this study mice were fed with sausages incorporated with various concentrations of ( 2%, 4%,and 6% (w/w) curry leaf powder for 4 weeks. Beef sausage incorporated with 6% w/w curry leaf powder significantly reduced the blood cholesterol level in albino mice. However, blood cholesterol levels estimated in mice fed with the 2% w/w and 4% w/w of curry leaf incorporated sausages were not significantly reduced but weight of abdominal adipose tissues was found to be significantly decreased, According to proximate analysis of beef sausage, crude fibre content was significantly high and fat content was significantly low in the 4% and the 6% w/w curry leaf incorporated sausages as compared to plain sausage. Thus from these observations it was concluded that certain bioactive compounds present in curry leaf might be responsible for the observed hypolipidemic effect as well as dietary fibres.
in feed might had reduce blood cholesterol by decreasing absorption of cholesterol or fatty acids and biliary cholesterol or bile acids in gastro intestinal tract [392].

Number of studies have reported effects of various types of extracts of MK on lipid profile In one such study involving administration of aqueous extract to alloxan induced diabetic rats for 8 weeks at dose of 600 mg /kg p.o total Cholesterol, LDL, VLDL, TG and plasma lipoproteins were significantly reduced whereas serum HDL levels remained unchanged. However another study reported that on administration of aqueous extract of *Murraya Koenigii* leaves at comparatively lower doses such as100,150 and 200 mg /kg p.o. for a period of 7 days in alloxan induced diabetic rats and normal rats did no significant alterations in levels of plasma total Cholesterol, HDL and LDL levels were observed [393,394]. Ethanolic extract of seeds and leaves at doses of 100 & 200 mg / kg p.o., also produced similar effects on lipid profile. On comparison between the two extracts seed extract was found to be exerting significantly higher hypolipidemic effect than leaf extract. This study further involved isolation of sapogenins from both the extracts and assessing their effect on lipids. The isolated sapogenins at selected doses (50 and 100mg /kg p.o.) decreased TG and LDL levels in trition induced hyperlipidemic rats[395]. These findings of the study concluded that saponins and saponin like compounds present in MK might had prevented cholesterol absorption by interfering with its entero-hepatic circulation and thereby increasing its faecal excretion [396]. Which might be responsible for the observed hypolipidemic effect of plant. When studies were carried out in diabetic ob/ob mice for 10 days at dose of 80 mg /kg p.o which was lower than previously tried doses, significant reduction in blood cholesterol level from 277 6 mg/dl on day 0 to 182.0 mg/dl on day 10 was along with reduction in body weight of treated animals thereby ascertaining the clinical utility of curry leaf in the management of high cholesterol level [397].

Birari et al (2010) found that continuous supplementation of the dichloromethane and ethyl acetate extracts of *Murraya koenigii* leaves for 2 weeks at 300 mg/kg/day p.o. significantly decreased the weight gain as compared to the body weight gain observed in rats fed with high fat diet only. Dichloromethane extract was found most effective as compared to ethyl acetate extract. After the treatment plasma Triglycerides and Total Cholesterol (TC) levels were significantly decreased while glucose levels were not altered significantly. In this study phyto constituent Mahanimbine when tested in vivo for assessing it’s antiobesity and lipid lowering ability at dose 30 mg/kg/ day p.o was found to exhibit significant decrease in body weight gain and decrease in the TC and TG levels [398].
2. Hypoglycemic effect:

*Murraya koenigii* is also found to be studied extensively for its hypoglycemic effect. On basis of available enormous research data indicating hypoglycemic effect of MK it can be considered as a promising herbal remedy for treatment of diabetes and may be prescribed as adjunct to dietary therapy and/or drug treatment for prophylactic control of Type II Diabetes. It was observed that feeding of the diet containing curry leaves at various concentrations of 5, 10 and 15% w/w to normal rats for 7 days and to alloxan induced mild diabetic and Streptozocin induced moderate diabetic rats for 5 weeks produced varying hypoglycemic and anti-hyperglycemic effect. Value of blood glucose observed at intervals of 1st, 2nd, 3rd and 4th week were found to be decreased in all the treatment groups whereas in diabetic control groups were found to be maintained stable. Highest reduction in blood glucose was seen in group fed with *Murraya Koenigii* diet of 15%w/w. Though the percentage reduction in hyperglycemia was dose dependent it was not statistically significant. However the reduction in blood glucose observed at 5th week was statistically significant at 10,%w/w and 15,%w/w diet no plateau effect was observed with any one of the treatment. In antihyperglycemic studies involving STZ diabetic rats blood glucose values in STZ diabetic rats fed with *Murraya Koenigii* diet decreased only marginally at all time intervals although significant regain in body weight was observed. Thus it was concluded that observed hypoglycaemic effect of *Murraya koenigii* was probably due to its ability to prevent alloxan and STZ induced destruction of β cells of islets in the pancreas. Since MK has been already reported in many studies bearing significant antioxidant or free radical scavenging properties its consumption in the early diabetic state might play a preventive role [399,400]. In another study involving administration of aqueous extract of *Murraya koenigii* leaves at doses 100, 150, 200 mg/kg p.o. for 7 days also exhibited reduction in blood glucose level in a dose dependent manner in normal as well as alloxan induced diabetic rats. However it’s hypoglycemic effect was significantly higher in diabetic rats than in normal rats but was comparatively less than that of standard chlorpropamide [394]. It was observed that when aqueous extract of *Murraya Koenigii* was administered at doses 200,300 and 400 mg/kg , after 4 hours of oral administration there was improvement in glucose tolerance in Alloxan recovered subdiabetic and mild diabetic rabbits. In this study onset of hypoglycemic effect began at 2 h and reached to maximum at 4 h in all the groups. The effect was found dose dependent up to 300 mg /kg. however, the response was found to be decreased at higher dose of 400 mg/kg dose [400]. When ethanolic extract of *Murraya Koenigii*. Leaves was administered orally at a dose of
200g mg/kg for a period of 30 days it significantly reduced the levels of blood glucose, glycosylated hemoglobin, urea, uric acid and creatinine in STZ induced diabetic rats and stimulated release of insulin in plasma. The hypoglucemic effect of extract was found to be greater than that of standard oral hypoglycemic drug glibenclamide [401]. One more study with ethanolic extract of *Murraya Koenigii* at same dose and duration of treatment (Ethanolic extract, 200mg/kg, for 30 days) supported the findings of previous study. but in addition to estimation of blood glucose, glycosylated haemoglobin and insulin in diabetic rats it also involved testing of the effect of extract on levels of various glucose metabolizing enzymes. In which it was observed that on treatment with ethanolic extract activities of enzymes hexokinase and pyruvate kinase were significantly decreased and activity of lactate dehydrogenase in liver and kidney were increased thus suggesting ability of plant to induce better tissue utilization of glucose in diabetic rats. Gluconeogenic pathway is always activated in diabetic patients It’s regulators such as glucose-6-phosphatase, Fructose 1,6 diphosphatase were found to be increased in liver and kidney of diabetic rats whose levels were set back to normal after treatment and were comparable with insulin. It was also observed that excess glucose in blood was disposed effectively as level of Glucose -6 Phosphate dehydrogenase enzyme was found to be increased after the treatment. The extract was also found to exert a favourable effect on glycogenic pathway since glycogen content in diabetic group was increased with increase in levels of glycogen synthetase and decrease in levels of glycogen phosphorylase thus indicating a vital role of plant in maintaining peripheral glucose homeostasis[402].

In another study chronic administration of aqueous (600mg/kg) and methanol (200mg/kg) extracts of leaves of *Murraya koenigii* for 8 weeks in alloxan induced diabetic rats was found to produce significant hypoglycaemic effect from very first week till 8th week of treatment where as significant rise in plasma insulin concentration was observed on 43rd and 58th days of study thus authors predicted the possibility of increased glycogenesis or decreased glycogenolysis or gluconeogenesis and/or insulin secretogogue effect on treatment with *Murraya koenigii* which might had increased glucose uptake and its utilization by cells[403]. In one more study methanolic extract administered at dose 500mg/Kg p.o. for 14 days exhibited significant hypoglycaemic effect[397]. Another study revealed that use of fresh leaves instead of dry powder is more effective in controlling diabetes induced hyperglycemia. This hypoglycemic effect of fresh curry leaves was thought to be due to presence of high content of essential oil and antioxidants[404]. *M. Koenigii* at dose 80mg/kg i.p for 10 days of
treatment was also observed to exert significant hypoglycaemic effect in diabetic obese mice [394]. Khan et al form his observations of curry leaf on carbohydrate metabolism concluded that curry leaf can exhibit a promising role in the treatment of diabetes. Hepatic glycogen and glycogenesis were increased, as evident from the increased activity of glycogen synthetase, and glycogenolysis. Gluconeogenesis was found to be decreased as evident from the decreased activity of glycogen phosphorylase and gluconeogenic enzymes [405].

Glucosidase inhibition such as α-amylase inhibition is another important target in the management of Post Parandial Hyperglycemia (PPHG) in diabetic patients. Chloroform extract of Murraya koenigii, was found to produce Porcine Pancreatic α-Amylase enzyme inhibition and Murine Pancreatic Glucosidases enzyme inhibition while M. koenigii methanolic extracts showed Murine Small Intestinal Glucosidases enzyme inhibition. Inhibition of these key enzymes of carbohydrate metabolism exhibited by Murraya koenigii may be responsible in retarding glucose absorption and thus suppressing PPHG [406].

In another study when tested for the effect of MK on diabetes induced body weight reduction in STZ induced diabetic rats it was found that treatment with aqueous extract 500 mg/kg p.o. for 15 days a prevented fall in body weight along with significant hypoglycaemic effect which was comparable with standard glibenclamide. In histopathological studies all the constituent structures of pancreas and kidney tubule in streptozocin induced diabetic group treated with extract were observed to be well maintained [407]. However, one study reported the failure of the hypoglycemic activity of Murraya koenigii in STZ induced type 1 diabetic rats [408]. Methanolic extract and nine carbazole alkaloids isolates of M. koenigii leaf, when subjected to an INS-1 cell line were found to inhibit insulin release in vitro, Girinimbine koenimbine, and murrayquinone-A were the most active among them thus indicating them useful in insulin resistance which is a peculiar problem observed with type II diabetes. Chronic administration for 14 days was found to produce significant reductions in serum glucose, urea, ALT and AST, etc. confirming the hypoglycaemic activity along with hepatoprotective effects [409]. It was also observed that when ethanolic extract of Murraya Koenigii was administered to STZ induced diabetic rats resulted in an increase in insulin and C-peptide levels and glucose tolerance. It was also observed that specific binding of [(125)I]-labelled insulin to the receptor was decreased in diabetic rats treated with extract along with a concomitant significant decrease in the levels of blood glucose, glycosylated haemoglobin and urea. The activities of carbohydrate-metabolising enzymes such as hexokinase, glucose-6-phosphate dehydrogenase and glycogen synthase in diabetic rats were significantly
increased near to normal in treated rats. The increased activities of lactate dehydrogenase, fructose-1,6-bisphosphatase, glucose-6-phosphatase and glycogen phosphorylase in STZ diabetic rats were significantly reduced following treatment with the MK extract [410]. Thus from all these observations MK was found to be effectively controlling glucose homeostasis in diabetic patients thereby assuring a promising role in management of Type II diabetes.

3. Hepatoprotective effect:-

While studying the effect of *Murraya koenigii* on liver it was observed that acute administration of methanol extract of *Murraya koenigii* at the dose of 500mg/kg p.o. significantly reduced serum albumin, globulin, urea, glucose, total protein, aspartate transaminase (AST) and increased cholesterol and alanine transaminase (ALT) indicating hepatic injury. But on chronic administration for 14 days urea, bilirubin ALT and AST levels were reduced showing hepatoprotective effects on prolonged use [397]. In another study on feeding of 10% curry leaf diet to rats produced no significant alterations in alkaline phosphatase level in liver. Even on histopathological studies no evidence of necrosis, degeneration or regeneration or bilestasis was observed indicating no deleterious effects of *Murraya koenigii* on liver. In one more study of *Murraya koenigii* bark extracts prepared in various organic solvents reported that the acetone extract of bark of *Murraya koenigii* exhibited significant ability to reduce the CCl4 induced increase in various of biochemical parameters like SGOT, SGPT, alkaline phosphate and total bilirubin level thus clearly indicating hepatoprotective activity of bark of *Murraya koenigii* [411].

4. Antinflammatory effect:-

Anti-inflammatory activity was studied for methanolic extract of MK using both in vivo and invitro methods. In invitro studies extract showed membrane stabilising effect and ability to inhibit thermal protein denaturation in dose dependent manner. When subjected to various antiinflammatory models like carrageen induced rat paw edema and Histamine and serotonin induced rat paw edema, methanol extract of *Murraya koenigii* inhibited paw edema in dose dependent (100, 200, 400 mg/kg p.o.) manner with maximum inhibition at dose of 400mg/kg p.o. It also showed dose depend protection from castor oil induced diarrhea. and cotton pellet induced granuloma thus ensuring efficacy of *Murraya koenigii* methanolic extract in inhibiting increase in no of fibroblasts, collagen and mucopolysaccharides synthesis during granuloma tissue formation. When tested for its effect on acetic acid induced increased vascular permeability in mice it was observed that at dose of 100 and 200 mg/kg p.o there
was moderate inhibition of vascular permeability while at dose 600mg/kg p.o the significant inhibition (60.02%) in vascular permeability was observed. Thus indicating the ability of *Murraya koenigii* extract to supress exudative phase of inflammation [412].

5. **Immunomodulatory effect:**

*Murraya koenigii* was also evaluated for its immunomodulatory effect and it was observed that methanolic extract of *Murraya koenigii* leaves showed significant increase of 24% and 56% in NO (nitrite) production from peritoneal macrophages at 416 μg/ml and 8.34 μg/ml respectively thus indicating increased phagocytic activity of macrophages in treated groups. In invivo studies with methanolic extract of leaves of *Murraya koenigii* phagocytic index was increased at dose of 500 mg/kg p.o administered for 20 days to albino mice. In group treated with *Murraya koenigii* at dose 250mg/kg p.o along with cyclophosphamid showed marginal reduction in WBC levels where as group treated with 500 mg/kg p.o. of *Murraya. koenigii* extract showed significant increase in levels of WBCS thus indicating the ability of extract to restore myelosupression caused by cyclophousphamide. Dose of 500mg/kg (p.o) produced increased antibody titre in response to ovalbumin reflecting an overall elevation of humoral immune response But no dose showed any effect on delayed type hypersensitivity reaction (DTH).determined by challenging left hind foot pad of mice with ovalbumine indicating that extractt has no stimulatory effect on T lymphocytes especially T\(_{DTH}\) lymphocytes and thus no effect on cell mediated immunity[413].

6. **Hematological effect:**

It was observed that on feeding rats with curry leaf diet (10% w/w/) levels of sodium and potassium in blood were found to be normal. and extracellular fluid volume was maintained. Total plasma protein and blood urea were unaltered and there was no influence on fibrinogen and antithrombin III concentration in blood [414].

7. **Antioxidant :-**

In rats fed with inclusion of 10% curry leaf in high fat diet it was observed that levels of hydroperoxides, conjugated dienes and free fatty acids in the liver and heart of rats were lowered along with increased activities of superoxide dismutase , catalase and glutathione transferase. Activities of glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase were also found to be increased in the liver and the concentration of glutathione was decreased. Thus it was concluded from these observations that
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supplementing a high fat diet with 10% curry leaf can prevent the formation of free radicals and maintain the tissues at normal[414].

oxidative stress is defined as disturbance in the balance between production of reactive oxygen species (ROS) and antioxidant defense system which is a common end point of chronic diseases such as diabetes as it causes reduction in oxidant status. Thus diabetic model is very commonly used. *Murraya koenigii* leaves extract were found to exert a protective effect against B cell damage and altered antioxidant defense system of plasma in streptozocine induced diabetic rats. It was found that altered levels of glucose glycosylated hemoglobin, insulin, TBARS, enzymatic and non enzymatic antioxidants were reverted back to near control levels after treatment with *Murraya koenigii* leaves extract. Protective effect of *Murraya koenigii* treatment on pancreatic beta cells was also confirmed by histopathological studies. These observations suggested that *Murraya koenigii* can protect pancreatic B cell damage by decreasing oxidative stress in diabetes. Thereby exerting antidiabetic effect by promoting insulin secretion either from protected β cells or from regenerated β cells [415].

In another study effect of *Murraya koenigii* on ultrastructural changes in liver of streptozocine induced diabetic rats were observed. In this study defatted ethanolic extract of *Murraya koenigii* was administered to STZ induced diabetic Wistar rats at dose of 200mg/kg (p.o.) for 30 days. Level of blood glucose glycosylated haemoglobin and insulin on treatment were observed to be almost similar to Control group levels. Increased levels of AST, ALT and ALP in liver due to liver necrosis caused by toxicant streptozocine were also observed to be restored to control levels in treatment groups. Increased levels of TBARS and hydroperoxides which are an index of increased oxidative stress and cytotoxicity were significantly reduced in extract treated groups thus further supporting antioxidant effects. Altered levels of nonenzymatic antioxidants such as vitamin C and E, reduced glutathione were brought back near to control group levels in diabetic rats treated with ethanolic extract. Decreased levels of SOD, CAT in the liver of diabetic rats were reverted back to the near normal levels by *Murraya koenigii* indicating its indirect antioxidant nature and the possibility of free radical scavenging activity. This observed antioxidant effect was suggested due to the presence of carbazole alkaloids, triterpenes and phenolics in leaf extract. Histopathological examination of liver in groups treated with *Murraya koenigii* extract depicted normal nucleus, nuclear membrane, and increased levels of glycogen thus indicating hepatoprotective effect of
Murraya Koengii possibly exerted due to its antioxidant potential against cytotoxic effects of streptozocin [416].

On administration of methanolic extract of Murraya koenigii leaves at doses of 100,200 mg/kg p.o. administered for 21 days in rats pre treated with CCl4 attenuated increase in transaminase (AST,ALT) enzymes activity suggesting the ability of Murraya koenigii leaves to accelerate regeneration of parenchymal cells. and protect them against membrane fragility thus preventing leakage of these enzymes. It was also observed that treatment attenuated increased levels of cholesterol and triglycerides in liver tissues. Stabilization of serum bilirubin, total protein and albumin level supported ability of MK to improve functional status of hepatocytes. Antioxidant and protector component such as SOD, GHS, catalase Vitamin C were observed near to normal in group treated with plant extract conjointly with CCl4 and this observed effect was thought to be occurring due to antioxidant activity of carbazole alkaloids and minerals such as Zn through scavenging of free radicals generated by CCl4. Thus once again indicating hepatoprotective activity of Murraya Koenigii. In invitro studies acetone, alcohol and aqueous extracts of Murray Koenigii were evaluated for DPPH radical scavenging activity where all 3 extracts exhibited strong antioxidant activity with IC50 values of 4.72μg /ml, 4.10 μg /ml,4.46 μg /ml respectively . The observed antioxidant effect was suggested due to presence of phenolics and flavonoids in these extracts [417].

In another study to evaluate the effect of Murraya Koenigii on lipid peroxidation in various tissues Sprague dawley rats were maintained on 10% fresh curry leaf diet for 60 days. In this study a break down product of unsaturated fatty acid Malondialdehyde (MDA) was found to be decreased and protective antioxidant enzyme catalase SOD levels were found to be increased in liver kidney and heart. Serum Ceruloplasmin levels were found to be unaltered and glutathione levels in liver heart and kidney were decreased. Glutathione reductase, Glutathione peroxidase ,Glucose 6- Phosphate dehydrogenase levels were also increased in liver which were thought to be responsible for maintenance of levels of glutathione [418]. An excellent antioxidant property observed in various studies was thought to be applicable for its use as a preservative and one such study reported that ghee samples treated with 1% curry leaves during clarification was able to impart higher resistance to oxidation than those treated with a mixture of BHT (butylated hydroxy toluene), BHA (butylated hydroxyl anisole), due to the presence of naturally-occurring antioxidants. and thus it was concluded that the curry leaves at 1% concentration could be used instead of BHT and BHA for extending the shelf-life of ghee [419].
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The antioxidant property of leaf extract of *Murraya koenigii* in different solvents was evaluated on the basis of oil stability index (OSI) together with their radical scavenging ability against 1-1-diphenyl-2-picrylhydrazyl (DPPH). In this study, methylene chloride extract and the ethyl acetate soluble fraction of the 70% acetone extract significantly prolonged the OSI values comparable to those of α-tocopherol and BHT. Further, five carbazole alkaloids were isolated from the methylene chloride extract and their structures were identified to be euchrestine B, bismurrayafoline E, mahanine, mahanimbicine, and mahanimbine based on 1H and 13C NMR and mass (MS) spectral data. The OSI value of carbazoles at 110 °C were found to be decreased in the order euchrestine B and mahanine > α-tocopherol > BHT > bismurrayafoline E > mahanimbicine, and mahanimbine. It was concluded that compounds euchrestine B and mahanine contributed to the high OSI values. The DPPH radical scavenging activity for these carbazoles was found to be in the order of ascorbic acid > bismurrayafoline E > euchrestine B, mahanine and α-tocopherol > BHT > mahanimbicine and mahanimbine [420]. In vitro antioxidant studies of methanolic extract of *Murraya koenigii* showed 70-60% inhibition of linoleic acid and that of water extract was 67.7%. The TBA analysis of the methanolic plant extract at seventh day of storage showed 63% of antioxidant activity at 1200 ppm, and was found to be higher than the effect produced by BHT [421]. In another study when mahanimbine and koenigine were evaluated for their radical scavenging activities in the DPPH system it was observed that both the alkaloids at concentration of 50 ppm produced activity of 91.6% and 18.8% respectively. Thus, it was concluded that the radical scavenging activity of these compounds was due to their hydrogen-donating ability and was attributed to the oxygen substitution at C-6 and C-7 positions of the carbazole nucleus [422].

8. Neuropharmacological Effects

When *Murraya koenigii* was subjected for screening its nootropic potential at different age groups it was observed that treatment exhibited dose dependent reduction in transfer latency and also significant reversal of memory deficit induced by Scopolamine and Diazepam in extroceptive behavioral models such as Elevated plus maze and Hebb William maze. Time taken to reach to the reward chamber was reduced in both young and old rats after 30 days treatment with *Murraya Koenigii* along with feed. On basis of this observed effect on retention of learned task it was suggested that already claimed antiinflammatory antioxidant and hypolipidemic properties of the plant in various studies might be responsible for net memory enhancing effect of *Murraya Koenigii* [423].
In search of underlying mechanism for this claimed memory enhancing effect of plant another study was carried out in young and aged mice which were fed with a diet containing 2%, 4% and 8% w/w of MK leaves for 30 days. The treatment produced reduction in brain cholinesterase and cholesterol levels thus further suggesting that underlying mechanisms of action may be probably procholinergic activity and/or cholesterol lowering effect. This observed Nootropic effect indicated the possibility of use of MK in management of Alzheimers disease. In one more study Acetylcholinesterase (AChE) inhibitory activity of mahanimbine a carbazole alkaloid isolated from *Murraya koenigii* was tested in vitro by using Ellman's method. The IC50 values observed for the methanolic extract, petroleum ether extract, chloroform extract and mahanimbine were 149.96 μg/ml, 69.91 μg/ml, 119.74 μg/ml and 83.11 μM respectively. Galantamine was used as standard exhibited IC50 value of 1.27 μM. Thus results revealed that the petroleum ether extract and its chemical constituent's mahanimbine produced better inhibitory effect on AChE thus supporting the already claimed possibility of therapeutic efficacy of MK in management of Alzheimer[424].

9. Antimicrobial effect:

Essential oil obtained from leaves of *Murraya Koenigii* by water and steam distillation was found to have antibacterial activity against *Bacillus subtilis, staphylococcus aureus, Corynобacterium pyogenes*, at the dilution of 1:500 and was also effective against *Proteus vulgaris* at the dilution of 1:250 [425]. Carbazole alkaloids mahanimbine, murrayanol and mahanine obtained by bioassay guided fractionation of the acetone extract of the fresh leaves of *Murraya koenigii* were observed to be mosquitocidal and antimicrobial and exhibited topoisomerase I and II inhibition activities. Pyrranocarbazole alkaloids grihmbine and mahanimbine were found to be effective against *Microsorum gypseum, Trichophyton rubrum Nocardia asteroidis, Candida albicans* where as Murrayanine 3 formyl-1 methoxycarbazole was found to be active against *Microsorum gypseum*, and candida albicans where as no alkaloid was found to be active against *Epidermophyton floccosum* [426]. Whereas in study carried with aqueous and ethanol extract of *Murray Koenigii* for anticandidial activity against *candida albicans* no significant effects were observed. In another study involving evaluation of antibacterial activity of aqueous and methanolic extracts of *Murraya koenigii* against 12 clinical and pathogenic bacterial strains isolated from aquatic animals, Methanolic extract of *Murraya koenigii* showed no effect against the tested bacterias; however, aqueous extract of the plant showed antibacterial activities against *V. alginolyticus, V. harveyi, V. vulnificus, A. hydrophila, C. freundii* and *S. putrefaciens* [427,428]. In one more study various extracts of
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*Murraya koenigii* Linn (Rutaceae) were screened for their *in vitro* antibacterial activity by agar diffusion method using standard antibiotic penicillin. The antibacterial activity of petroleum ether, chloroform, acetone, methanol and aqueous extract of leaves of the plant were studied at the concentration of 10, 25, 50, 100, 250, 500 and 1000 μg/ml. All the extracts at various concentrations showed antibacterial activity equivalent to that of standard against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* as test organisms. Chloroform acetone and methanol extracts were reported to show better activity than the standard against all four micro organisms. Aqueous extract was observed to be more effective against *Bacillus subtilis* and *Staphylococcus aureus*. Whereas Petroleum ether extract was more effective against *pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* [429]. Recently one more study reported that a monomeric protein, designated as APC, isolated from *Murraya Koenigii* had shown potent antibacterial activity against all the human pathogenic strains such as *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Bacillus subtilis*. The inhibition observed was comparable to that of commercial antibiotics chloramphenicol, streptomycin and gentamycin with MIC values ranging from 13 to 24 μg/ml. These observations thus encourage to explore MK as a potential antibacterial herb [430]. When screened for antiviral effect of ethanolic extract of whole plant and only bark, both of them failed to produce any significant antiviral effects when tested against proton virus X [431].

10. Antiemetic and Anti diarrhoeal:

Juice of tender leaves MK when taken orally is found to be helpful in arresting vomiting [432]. (98) (95) In study carried by S. Mandal et al. the carbazole alkaloids, koenimbine and kurrayam, isolated from the seeds of *M. koenigii*, when administered orally to rats, exhibited significant and dose-dependent antidiarrhoeal activity in rats at doses 30 and 50 mg/kg (p.o). The isolates were found to decrease the propulsion of charcoal meal through the gastrointestinal tract and the PGE2-induced entero pooling significantly in rats. The observed effects were found to be comparable with standard Diphenoxylate supporting the traditional use of MK in the management of diarrhoea [433].

11. Oral hygiene

The cold extract of curry leaves of pH of 6.3 to 6.4 were found to be rich with Chlorophyll and had been proposed to reduce halitosis. It was observed that there was a decrease in the bad breath in 10 male volunteers (age 30-52 yrs) after holding the curry leaves in the mouth
for 7 minutes. It was suggested that fresh curry leaves have 2.6% essential oils containing various sesquiterpenes possessing antibacterial and antifungal activity. It was suggested that essential oils in curry leaves being soluble in water dissolves in saliva and the terpenes present in them might exert antibacterial activity. Folic acid present acts as a mouthwash and also reduces the severity of gingivitis [434]. Terpenes present in curry leaves have been found to reduce airborne chemicals and bacteria. Essential oils, chlorophyll, beta-carotene and folic acid, riboflavin, calcium and zinc present in leaves were thought to be acting on the oral tissues to aid in keeping up good oral health. It was observed that chewing of 2 to 4 fresh curry leaves with 10 to 15 ml water in the mouth, swishing for 5 to 7 minutes and rinsing the mouth out with water can be of help in keeping good oral hygiene [435]. All these proposed mechanisms and observations suggest the use of curry as a cheap herbal mouthwash.

12. Hair growth promoting activity

*Murraya koenigii* (Rutaceae) was tested for their hair growth promoting activity in a concentration range for 1-10% w/v. The oil containing 7% and 8% concentration of *M. koenigii* showed early initiation of hair growth follicle in 6 days with increase in number of hair follicles [436].

13. Insecticidal activity:

The use of conventional insecticides has raised concern about their threat to the environment and development of insecticide resistance in insects is also another concern hence there is an imperative need for the development of safer, alternative crop protectives such as botanical insecticides and antifeedants. *Murraya koenigii* has been evaluated for these properties in various studies. Essential oils and methanolic extract of *Murraya koenigii* (L.) Spreng were tested for their insecticidal activities against adults of two insects *Sitophilus oryzae* (L.) and *Callosobruchus analis* (L.), using direct contact application method in which potent insecticidal activity within a day after treatment was observed for essential oil obtained from leaf, for both the insect species, while methanol extract showed 100% mortality against *S. oryzae* after 3 days treatment. In conclusion, methanol extract of *M. koenigii* and essential oil obtained from Murraya can be utilized for managing field populations of *S. oryzae*, and *C. analis* [437].

In another study dealing with the evaluation of efficacy of some leaf powders via free choice and no choice assay against infestation of chick pea seeds by the pulse beetle *Callosobruchus*
chinesis during storage, Murraya koenigii was found to be the most effective in reducing the orientation, oviposition and causing the mortality of bruchids at dose of 2% (w/w). The study demonstrated that plant powder can play an important role in protection of chickpea from insect invasion during storage[438]. Antifeedant and toxic effects of leaf extract of Murraya koenigii were evaluated with crude acetone extract against the tobacco cut worm, Spodoptera litura Fab. and the castor semilooper, Achaea janata L. (Noctuidae: Lepidoptera) and was found to produce moderate effects towards these pests. Thus plant extract possess potential for use as alternative crop protective against certain pest species [439]. Volatile oil of Murraya koenigii leaves was found effective when evaluated in the laboratory for contact and fumigant toxicity against Callosobruchus chinensis developing in green gram and chickpea seeds [440]. In one more study acetone extract of Murraya koenigii Linn. was found to be effectively ovicidal against the eggs of C. chinensis [441].

14. Trypsin inhibitory activity

Gahlot et al reported the possibility of trypsin inhibitory activity of α- β protein present in Murraya koenigii. The inhibitory activity gradually decreased with increasing temperature and was completely lost at 90°C. It was observed that below pH 7.5 it precipitates whereas at high temperature, it was found to be structurally stable but not functionally stable. Correlation of decrease in inhibitory activity and helical content at increasing temperatures suggested a possible role for alpha helical structure in inhibitory function of trypsin. [442].

15. Cytotoxic activity

When tested for cytotoxic effect chloroform extract of roots and bark together of Murraya koenigii, and the isolates Koenoline and Murrayanine exhibited ED50 values of 6μg/ml, 4μg/ml and 26μg/ml respectively, in κβ cell culture test system [346]. Phyto constituents such as Girinimbine and Mahanimbine were also reported to exhibit cytotoxic activity towards cultured κβ cells. Whereas in another study Mahanimbine and Koenimbine alkaloids isolated from leaves of Murraya Koenigii were reported without any indication of antitumor activity when tested [443]. It is also reported that 9formyl-3 methyl carbazole obtained from roots of Murraya koenigii displayed weak cytotoxic activity. In study carried by Ito et al three carbazole alkaloids, mahanine pyrayafoline-D and murrayafoline-I were found to produce significant cytotoxicity against HL-60 cells by inducing apoptosis activation of the caspase-9/caspase-3 pathway in a time-dependent manner [444]. In a study carried out by Bhattacharya et al, Mahanine isolated from the leaves of Murraya koenigii was found to
produce dose and time-dependent anti-proliferative activity in acute lymphoid and chronic myeloid leukemic cell lines [445].

16. Toxicity studies:

During acute toxicity studies no mortality was reported for aqueous extract and methanolic extract(79) of *Murraya koenigii*. However another study was found to be reporting that methanolic extract of *Murrayakoenigii* was moderately toxic (LD50=316.23mg/kg) at acute dose of 500 mg/kg (p.o) and at higher doses caused liver inflammation but showed little effect on haematology and relative organ weight of lung heart and spleen. But the same study also claimed that on chronic administration for 14 days extract exhibited significant hypoglycemic and hepatoprotective effects [375]. (40)

2.3.2. *Ocimum Sanctum* (Krishna Tulsi)

*Ocimum sanctum* is held sacred by Hindus all over India and is frequently grown in courtyards and temples. The plant is propagated by seeds. In India, the plant is grown throughout the country from Andaman and Nicobar islands to the Himalayas up to 1800 meters above the sea level [446]. It is also abundantly found in Malaysia, Australia, West Africa and some of the Arab countries.

The plant is part of Ayurveda and Unani system of medicine and is well recognized in Ayurveda for its medicinal properties [446]. In Ayurveda Tulsi (*Ocimum sanctum* L.) has been well documented for its therapeutic potentials and is described as Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna) [447].

**Macroscopic**

Tulsi plant is erect much branched, softly hairy around 30-75 cm in height. It is found all over India up to 1800 m in the Himalayas and also in Andaman Nikobar islands. Leaves are 2.5 -5 cm long and 1.6 -3.2 cm wide elliptic oblong, acute or obtuse, entire or serrate, pubescent on both the sides, minutely gland dotted. Flowers are purplish or crimson in racemes, close whorled about 3 mm long and broad, pedicles longer than calyx, slender shortly apiculate, lower lip is longer than upper having four mucronate teeth, lateral two short and central two largest corolla about 4mm long. Fruits are in a group of 4 nutlets, each with one seed, enclosed in an enlarged membranous veined calyx, nutlets are sub globose or broadly ellipsoid, slightly compressed, nearly smooth, pale brown or reddish with small black markings. seeds are rounded to oval, brown, mucilaginous when soaked in water, 0.1 cm long, slightly notched at the base, no odour, pungent and mucilaginous in taste [448,449]
Synonyms
Sanskrit – Krishna Tulasee
Bengali-Tulsi
Gujarati-Tulsi
Hindi – Tulsi, Kala Tulsi
Malyalam-Tulasi
Marathi-Tulas
Tamil-Thulasi
Telagu – Tulasi, Krishna Tulasi, Kari Tulasi, Sri Tulasi

*Ocimum sanctum*

**Phytochemistry:**
Chemical investigations by GLC essential oil of the leaves revealed presence of volatile oils with phenolic compounds like eugenol and β-Caryophyllene as the major constituents. Other compounds identified were nerol, methyl ester, Caryophyllene, terpinene, gama selinine, alpha pinine, beta pinine, camphor and carvacol. The flavones present include apigenin and luteolin, the flavone O-glycosides apigenin 7-O-glucuronide, luteolin 7-O-glucuronide, the flavone C-glucosides orientin and molludistin, and ursolic acid were found to be present [450, 451, 452, 453]. Number of sesquiterpenes and monoterpenes present include bornyl acetate [453, 454], β-elemene, methyl-eugenol, neral, β-pinene, camphene, α-pinene [451] etc, ursolic acid, campesterol, cholesterol, stigmasterol sitosterol ad methyl esters of common fatty acids were found to be present as minor constituents [455]. One of the study had also suggested the possibility of the isolation of a high content of phenolic acid i.e. rosameric acid from invitro callus cultures than field grown plant organs of holy basil [456]. Essential oil of *Ocimum sanctum* was found to contain monoterpenes like α-thugene, camphene, sabinene,
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β-pinene, limonene, oxygenated monoterpenes such as linalool and borneol, sesquiterpenes including α-copanene β-elemene, β-caryophyllene, α-humulene, γ-muurolene, α-bulnesene and phenyl propenes including eugenol and methyl eugenol [457]. Besides the volatile oil plant is reported to contain alkaloid, glycosides, saponons and tannins. The leaves contain Ascorbic acid and carotene [458]. Presence of Two phenylpropane glucosides had been detected which were identified as 4-allyl-1-O-/β-o-glucopyranosyl-2-hydroxybenzene and 4-allyl-i-O-fl-o-glucopyranosyl-2-methoxybenzene (eugenyl-β-D-glucoside) [459]. Leaves were found to contain 0.15% Ca++, 0.34% Phosphorus along with insoluble oxalates.

**Chemical Structures:**

- **Eugenol**: ![Eugenol](image)
- **Methyl Eugenol**: ![Methyl Eugenol](image)
- **Methyl Chavicol**: ![Methyl Chavicol](image)
- **Beta Ocimene**: ![Beta Ocimene](image)
- **Neral**: ![Neral](image)
- **Geranial**: ![Geranial](image)
Pharmacological effects:

1. Anti bacterial effect:
The essential oil was reported to possess antibacterial and insecticidal properties. The oil has been shown to have inhibitory effects on growth of *Mycobacterium tuberculosis* and *Micrococcus pyogenes* var. *aureus*. It was found to exhibit one tenth anti-tubercular potency of streptomycin and one-fourth that of isoniazid [460]. Aqueous and acetone extracts of *Ocimum sanctum* were also found to be effective against many plant fungi, *Alternaria tenuis, Helminthosporium spp,* and *Curvularia penniseli* [461]. Essential oil of *Tulsi* was tested on plant pathogenic fungi as well (*e.g.* *Alternaria solani, Candida guillermondii, Colletotricum capsici, Curvularia spp, Fusarium solani, Helminthosporium oryzae* and the bacterial strains, *Anthrobacter globiformis, Bacillus megaterium, Escherichia coli, Pseudomonas spp, Staphylococcus aureus, Staphylococcus albus* and *Vibriocholerae* [462,463]. The essential oils of *Tulsi* has been reported effective against both Gram positive and Gram-negative bacteria and were comparable with the effectiveness of clove oil [464,465].

Antimicrobial activity of *Ocimum sanctum* was found to be higher as compared to commonly available other species of *Ocimum* (*i.e.* *O. canum, O. gratissimum, O. basilicum*) in India [466]. Aqueous extract, alcoholic extract and seed oil of *Tulsi* had also shown antimicrobial properties against enteric pathogens [467,468]. It also exhibited significant antimicrobial activities against some of the clinical isolates and multi-drug resistant *Neisseria gonorrhoeae* [469,470]. The ethanolic extracts have ability to inhibit β-lactamase producing methicillin-resistant *Staphylococcus aureus* and methicillin–sensitive *Staphylococcus aureus* [471]. Essential oil of *Ocimum sanctum* reported to have shown antimicrobial activity against *Propionibacterium acnes* in in-vitro study and minimum inhibitory concentration (MIC) value found to be 3.0% v/v [472]. Essential oil obtained from fresh leaves was found to exert more effective antimicrobial effect than that exerted by oil extracted from dry leaves whereas exactly reverse effect was observed when tested against fungal infections [473]. Herbal preparations containing *Ocimum sanctum L.* have been suggested to shorten the course of illness, clinical symptoms and biochemical parameters in patients suffering from viral hepatitis. Aqueous extract of Tulsi is found effective in patients suffering from viral encephalitis [474].

As a prophylactic against malaria, fresh Tulsi leaves have been reported to be taken with black pepper in the morning. Ayurvedic preparation containing *Ocimum sanctum L., Allium stivum, Piper nigram* and *Curcuma longa* has been shown to possess antimalarial activity.
against *Plasmodium vivax* and *Plasmodium falciparum* [474]. Also this preparation has been found to relieve the clinical symptoms in 52% of *Plasmodium vivax* patients and 100% of *Plasmodium falciparum* patients [474]. A decoction of the root of Tulsi plant is given as a diaphoretic in malarial fever [475]. As far as its antimalarial effect is concerned Tulsi extracts and essential oil

2. Hypoglycemic effect:

In Adult rabbits administration of fresh *Ocimum sanctum* leaves (1 and 2 g/day) for four weeks have been observed to exert significant hypoglycaemic and uricosuric effects [476]. Upon 15 days treatment with *Ocimum sanctum* leaf extract 43% reduction in blood sugar level were observed in experimental rats with diabetes mellitus induced by alloxan. On termination of feeding with fresh leaves of *Ocimum sanctum* blood glucose levels were found to be increased indicating that the fresh leaves possess hypoglycaemic properties [477]. Similarly, oral administration of ethanolic extract of *Ocimum sanctum* to the rats with diabetes, induced by streptozotocin, showed reduction in serum glucose level. This reduction was 91.55% and 70.43% in normal and diabetic rats respectively, where Tolbutamide was used as a standard[478]. Dry *Ocimum sanctum* leaf powder when fed at 1% of total diet for 30 days to the rats with diabetes induced by alloxan, fasting blood sugar, total amino acids, total cholesterol, triglyceride, phospholipids and total lipid levels were found to be reduced significantly [479]. Similarly, administration of methanolic extract of *Ocimum sanctum* at a dose of 200 mg/kg, p.o for 30 days produced increase in activities of glucokinase and hexokinase significantly [480]. Where as administration of seed oil of *Ocimum sanctum* *at dose* 800 mg/kg,(p.o) /day to experimentally induced hyperglycaemic and hypercholesterolaemic rabbits for four weeks was found to reduce cholesterol levels significantly with no significant effects on blood sugar level [481]. *Ocimum sanctum* extract at dose 500mg/kg p.o. was found to reduce blood glucose and oxidative stress in rats with streptozotocin-induced diabetes [482]. It was also found that feeding of aqueous extract (200 mg/kg p.o )of whole *Tulsi* plant for 60 days significantly delayed insulin resistance in fructose fed experimental mice [483]. The alcoholic extract and other organic solvent fraction’s extract has been found to stimulate insulin secretion from perfused rat pancreas, isolated islets and clonal pancreatic β-cells. From these observation the authors proposed the possible mode of action for *Ocimum sanctum* that it might had stimulated adenylate cyclase/ c AMP or the phosphatidyl inositol or might had exerted direct effect on exocytosis inducing mobilization of intracellular Ca++ along with promoting Ca++ entry thereby leading to the secretion of insulin [484]. In one of the initial randomized controlled clinical trails, anti-
diabetic properties were studied in 40 non insulin dependent diabetes mellitus (NIDDM) patients. It was observed that consumption of dried Tulsi leaf powder made from 2.5 g of fresh leaves per day orally on empty stomach could reduce the fasting glucose and postprandial blood glucose [485]. In another trial on twenty seven NIDDM patients, it was observed that supplementation of Tulsi powder along with hypoglycaemic drugs for one month could significantly decrease the blood glucose, glycosylated proteins, total amino acids, uronic acid, triglycerides, low density lipoprotein (LDL) and very low density [486].

3. Anti-inflammatory effect:
The aqueous and methanolic suspension of Ocimum sanctum were found to inhibit acute as well as chronic inflammation in rats with carrageenan induced paw edema, croton oil induced granuloma and exudates, at a dose of 500 mg/kg, p.o /day [487]. The oils extracted from fresh leaves (essential oil 200 mg/kg) and seeds (fixed oil 0.1ml/kg,) of Tulsi have shown reduction in paw edema induced by carrageenan, serotonin, histamine and prostaglandin-E-2 exhibiting anti-inflammatory activity [488]. The mechanism of action for the observed anti-inflammatory effects of Ocimum sanctum was thought to be via cyclo-oxygenase and lipooxygenase pathways [489,490].

On comparison of the anti-inflammatory effects exerted by fixed oils of various species of Ocimum (O. sanctum, O. basilicum, O. americanum) Ocimum basilicum was found to be exhibiting maximum inhibition (72.42%), against phlogistic agent induced paw oedema. Whereas O. sanctum fixed oil containing 16.63% linolenic acid provided 68.97% inhibition.(48). Fixed oil of Ocimum sanctum was found to inhibit enhanced vascular permeability and leukocyte migration as evidenced by carrageenan induced inflammatory stimulus [489]. Extract of seeds from Ocimum sanctum were studied for anti-inflammatory effects of carrageenan, leukotrine and arachidonic acid induced paw edema in rats. Ocimum sanctum seed oil was found to produce maximum percentage inhibition of leukotrine induced paw edema [491].

4. Anti oxidant activity: When tested for evaluation of antioxidant effect it was observed that ethanolic extracts from Holy basil exhibited the highest antioxidant activity. Antioxidant activity of Holy basil was heat stable, even after heating at 80 °C for 60 min Antioxidant activity of Holy basil extracts was increased with increasing concentrations, ranging from 0.10 to 0.75 mg/ml and then reached a plateau at concentrations ranging from 0.75 to 1.0 mg/ml. In this study the ethanolic extracts of Holy basil exhibited strong superoxide anion scavenging activity, Fe2+ chelating activity, and reducing power in a concentration-
dependent manner, and additionally acted as radical scavengers and lipoxygenase inhibitors. Hence the Authors suggested that ethanolic extracts could be used as natural food antioxidants and possibly a good substitution for artificial antioxidants [492]. *Ocimum sanctum* pre treatment significantly prevented the rise in MDA levels and up-regulation of SOD activity, suggesting that *Ocimum sanctum* has an ability to prevent the excessive formation of reactive oxygen substrates (ROS) secondary to reperfusion injury. *Ocimum sanctum* did not show any effect on endogenous TSH levels, it prevented their consumption secondary to reperfusion injury. *Ocimum sanctum* significantly attenuated hypoperfusion-induced changes like microglial activation and also significantly attenuated the severity of cellular oedema and perivascular inflammatory infiltrate. On testing the effect on memory latency to reach platform of water maze was found to be improved in hypo perfused animals by *Ocimum sanctum* treatment [493]. In another study treatment with *O. sanctum* was able to prevent the elevation of lipid peroxidation in sub-acute exposure but not in acute noise exposure. *O. sanctum* treatment was also found to prevent the elevation of superoxide dismutase in all the durations of noise exposure treatment groups. *O. sanctum* extract was also found to prevent the noise induced increase in catalase levels and could significantly increase GSH levels. Ethanolic extract of *O. sanctum* produced DPPH radical scavenging activity, with an IC50 value of 28.89 mg/ml and nitric oxide scavenging activity, with an IC50 value of 26.92 mg/ml. The superoxide scavenging activity of *O. sanctum* extract was evident from the IC50 value of 27.63 mg/ml. Extract of *O. sanctum* also exhibited hydroxyl radical scavenging activity, with an IC50 value of 661.11 mg/ml and 2,2-Azinobis-ethylbenzothiozoline-sulphonic acid diammonium salt radical scavenging activity, with an IC50 value of 8.82 mg/ml [494].

### 5. Immuno modulatory Effect:

The fresh leaf of *O. sanctum* is consumed with the traditional belief that it enhances immunity. Various studies have been carried out to scientifically investigate this claimed effect of *Tulsi*. Rats treated with methanolic extract of *O. sanctum* when challenged with typhoid H-antigen and sheep red blood cells (SRBCs) exhibited a significant rise in antibody titre in both groups as compared to saline treated controls. In the Erythrocyte (E)-rosette formation test, it was observed that E-rosette formation in *O. sanctum* treated groups was significantly higher as compared to controls[495]. In another study steam distilled extract of fresh leaves of *O. sanctum* enhanced humoral immune responses in experimental rats. This was evident by enhanced count of anti-sheep red blood cell (anti-SRBC) haem agglutination titre and IgE antibody titre as measured by passive cutaneous anaphylaxis in rats. Antigen
(egg albumin) induced histamine release from peritoneal mast cells of sensitized rats in in-vitro was significantly inhibited by fresh leaves extract of *O. sanctum* [496]. Rats were immunized with SRBCs and when were treated with oil of *Ocimum sanctum* (3ml/kg/day, i.p.) for 6 days produced a significant increase in anti sheep-RBC antibody titre. Whereas on treatment with oil of *O.Sanctum* 13 days with same dose significant inhibition of antigen induced histamine release from peritoneal mast cells of rats sensitized with egg albumin along with Freund’s complete adjuvant and triple antigen was observed. Treated mice also showed significantly low migration inhibition (LMI). Oil of *O.Sanctum* was also found to produce synergistic effect when administered along with Diazepam. Animals pre treated with *Ocimum sanctum* oil was also found to cause significant attenuation of T-cell mediated response [497]. Thus *O. sanctum* seed oil was found to possess immuno modulatory potential as humoral and cellular immunity both were found to be increased in both non-stressed and restraint stressed rats. Bovine mastitis is a disease, usually caused to lactating bovine by bacterial infections, eventually damages the udder tissues. Aqueous extract of *O.sanctum* showed immunotherapeutic potential in bovine sub-clinical mastitis. Polymorphonuclear cells (PMNs) are the primary cellular defence cells of the mammary glands of the bovines and they are depressed during periparturient period. Use of antibiotics to treat mastitis further depresses the activity of PMNs. Infusion of aqueous extract *O.sanctum* (100 mg/teat/day) for seven days reduced total bacterial count (TBC) in the milk and increased neutrophil and lymphocyte counts with enhanced phagocytic activities and phagocytic index. Similarly, lysozyme content of the milk PMNs were also enhanced significantly in animals pre treated with *O. sanctum*. It was suggested that the bioactive constituents could be urosolic acid, oleanolic acid and sarigenin, which may possess immuno modulatory potential indicated by percentage increase in lymphocyte, enhanced activity of the phagocytosis of PMN cells in the bovine mammary gland, and the reduction in TBC in the milk [498]. Therapeutic efficacy of *O. sanctum* seed oil was also studied in twenty three confirmed cases of bovine mastitis in buffaloes. Where group treated with fixed Oil of *O.sanctum* recovered within 5 days whereas the group treated with antibiotic cloxacillin showed recovery within 4 days and the group treated with combination of oil and antibiotic was recovered within 3 days. Thus indicating the ability of tulsi oil to cure the bovine mastitis [499].

6. Effect on CNS:

*Ocimum sanctum* 2.5% ethanol extract when administered for 5 days consecutively was found to reduce the severity and duration of electroshock and pentylenetetrazole-induced convulsions and also decreased the apomorphine-induced fighting response and lowered
open-field activity. All these actions resemble the activity of low doses of the barbiturates. However *Ocimum sanctum* extract also caused an increase in stereotypic activity, i.e. ambulation, paper biting, circular movements, which is indicative of CNS stimulant/increased adrenergic activity such as that seen with the amphetamines. Haloperidol given alone to rats increased immobility time while sulpiride did not alter forced swimming immobility time. Haloperidol and sulpiride [500] when given with *Ocimum sanctum* extract fully blocked the effect of the latter, as seen in the behavioural despair test, suggesting a possible Dopaminergic receptor mechanism for *Ocimum sanctum* extract. Bromocryptine, a potent Dopamine receptor agonist [501] reduced the immobility duration and, when combined with *Ocimum sanctum*, there was an apparent additive action. This effect indicated similar dopaminergic activation [502]. *Ocimum sanctum* attenuated sciatic nerve transection, i.e., axotomy-induced mechanical hyperalgesia, tactile alldynia, increased spinal nociceptive sensation, motor in-coordination and axonal degeneration. Moreover, administration of *Ocimum sanctum* attenuated axotomy-induced raised levels of TBARS which is an index of lipid peroxidation, decreased levels of reduced glutathione (GSH), an endogenous anti-oxidant. Therefore, it was tentatively suggested that anti-oxidant action of *Ocimum sanctum* might had contributed significantly in preventing the induction of axotomy-induced neuropathy. Along with rise in oxidative stress, axotomy also led to an increase in calcium levels in sciatic nerve suggesting the key role of calcium in axotomy-induced neuropathy. *Ocimum sanctum* was found to decrease the calcium levels in axotomised animals. Therefore, it was proposed that *Ocimum sanctum*-induced decrease in oxidative stress and calcium levels may be responsible for ameliorating axonal degeneration and neuropathy in sciatic nerve transection model [503]. In the groups of rats pre treated with *Ocimum Sanctum* extract for 7 days and subjected to noise stress on 8th day it was observed that stress induced altered values of Acetyl choline (ACh) content and Acetyl choline esterase (AChE) activity were brought back to near control values in treatment groups [504].

**7. Anti-stress effect:**

The treatment with ethanolic OS extract was found to prevent the increase in dopamine (DA) and 5HT levels, caused by the exposure to three different durations of noise stress. In this study animals were subjected to acute, sub-chronic and chronic noise exposure at 100 dB intensity. From the results obtained it was proposed that OS might had acted by influencing either the activity of tryptophan hydroxylase or the reuptake of neurotransmitter [505]. In a study conducted on experimental animals by Sethi *et. al. Tulsi* fresh leaves were found to significantly reduce the effects of anemic hypoxia induced oxidative damage [506], it was
observed that on feeding of 2 g of fresh *Tulsi* leaves for 30 days, the hemoglobin, serum glucose and plasma malondialdehyde (MDA) levels remained significantly higher when anaemic hypoxia condition was induced.

In another study the ethanolic extract of whole plant of *Ocimum sanctum*, was found to increase physical endurance and even found to reduce the degree of occurrence of aspirin induced ulcer in mice. Ethanolic extract was also found to prevent carbon tetra chloride induced hepatotoxicity and leukocytosis due to injection of milk when administered at a dose of 100 mg/kg,[39]. Administration of ethanolic extract of *Ocimum sanctum* at a dose of 200 mg/kg p.o. for seven days increased production of adrenaline, noradrenaline, monoamine oxidase and caused decrease in dopamine and 5-hydroxytryptamine (serotonin) levels in mice subjected to forced swim stress [507] . One study was found to be conducted with the aim of exploring the possibility of antioxidant property of the drug for exerting antistress effect. In this study the alcoholic and aqueous extracts of *Tulsi* were found to inhibit lipid peroxidation of erythrocytic membrane in a dose dependant manner. The alcoholic extract produced greater inhibition (IC50 at 16 μg) as compared to aqueous extract (IC50 at 80 μg) [508]. Administration of ethanolic extract of *Ocimum sanctum* (100 mg/kg, /day, i.p.) for 15 days had a normalizing action on discrete regions of brain and controlled the alteration in neurotransmitter levels due to noise stress [509]. Ethanolic extracts of *Ocimum sanctum* at dose100 mg/kg (p.o) leaves prevented the elevation in plasma corticosterone levels following acute and chronic noise stress [510]. The mean swimming time of mice treated with ethanolic extract (400 mg/kg,p.o) of the roots of *Ocimum sanctum* was found to be increased significantly in forced swimming stress test [511].

Rats treated with petroleum ether extract of *Ocimum sanctum* 30 minutes before the pentobarbitone induced hypnotization at dose of 200 or 500 mg/kg p.o. were found to escape from watermaze with less number of errors [512]. The methanolic extract of *Ocimum sanctum* when given at a doses of 50 and 100 mg/kg (p.o) was observed to be significantly reducing the oxidative stress caused by ischemia-reperfusion injury, cigarette smoke, foot shock and iron overload hepatotoxicity [513]. Treatment with *Ocimum sanctum* essential oil was found to prevent restraint stress induced rise in levels of blood glucose , urea, lactate dehydrogenase (LDH) and alkaline phosphatase .Restrained stress induced increased membrane protein clusterization, fluidity and reduced membrane thickness of red blood corpuscles (RBCs)was also found to be reduced [514]. From all these observations it can be concluded that *Ocimum sanctum* can be used as an alternative herbal remedy to treat stress.
8. Anti-carcinogenic properties

The anti-carcinogenic properties have been evaluated in the experimental animals induced by different types of carcinogens. *Ocimum sanctum* leaves when fed to experimental rats with 600 mg/kg diet for ten weeks, significantly reduced the 3,4-benzo(a)pyrene [B(a)P] and 3’-methyl-4- dimethyl amino azobenzene (3’MeDAB) induced squamous cell carcinoma and hematoma incidences [515]. The anti-cancer activity of *Ocimum sanctum* had also been reported from Philippines where juice of fresh leaves was applied on the skin of experimental mice thrice a week for 20-minutes along with tumor promoter agents (dimethylbenzantracene as initiator and croton oil as promoter of cancer). No incidences of tumor were found in 20 weeks follow up period in *ocimum sanctum* treated group [516]. The ethanolic extract of *Ocimum sanctum* leaves at a dose of 400 and 800 mg/kg, have found to modulate carcinogen metabolizing enzymes such as cytochrome P-450, cytochrome-b5 and aryl hydrocarbon hydroxylase of mice liver [517].

9. Radio-protective properties

Aqueous extract of *Ocimum sanctum* was found to offer greater radio protection at optimum dose of 10 mg/kg i.p administered for consecutive 5 days before subjecting to whole body \( ^{\gamma} \) radiations (11 Gy) by increasing survival period of mice up to 30 days [518]. Among three plants extracts viz *Withania somnifera, Plumobogo rosa* and *Ocimum sanctum*, tested on experimental mice bone marrow survival following 2 Gy \(^{\gamma}\)-radiation, aqueous extract of *Ocimum sanctum* provided highest radioprotection as measured by an exogenous spleen colony forming unit (CFU-S) assay. It was also observed that the *Ocimum sanctum* extract had no toxic effects compared to synthetic radioprotector WR-2712 [519]. Radio-protection efficacy of two flavonoids, orientin and vicenin, isolated from leaves of *Ocimum sanctum* administered at dose of 10 mg/kg, /day i.p to mice for five days were compared with synthetic radio-protectect tor aminothiol, 2-mercaptpropionyl-glycerine ‘MPG’ (20 mg/ kg,), WR-2721 (150 mg/kg, bw). The experimental mice were subjected to whole body exposure to 2 Gy \(^{\gamma}\)-radiations for 30minutes and bone marrow chromosomal aberrations were studied. It was observed that vicenin provided maximum protection from radiation induced chromosomal aberrations and MPG the least, while orientin and WR-2721 provided almost similar effects [520].

WR-2721 and aqueous extract of *Tulsi* were found to produce synergistic effects when combined together [521]. The study was continued further for ensuring the ability of Tulsi to offer protection against radiations induced lipid peroxidation in liver of adult swiss mice.
Where aqueous extract of Ocimum sanctum (10mg/kg) was administered for 5 days consecutively followed with 4.5 Gy γ-radiation for 30 minutes and levels of glutathione (GSH) and the antioxidant enzymes glutathione transferase (GST), glutathione reductase (GSRx), glutathione peroxidase (GSPx), super oxide dismutase (SOD) and lipid peroxide (LPx) in the liver were estimated at different intervals post treatment. Significant reduction of the lipid peroxidation was observed in treatment groups as significant recovery of antioxidant enzymes to normal levels was also observed. From these observations the free radical scavenging capacity of flavonoids of Tulsi plant was proposed as possible mechanism for conferred radioprotection [522,523].

10. Contraceptive studies

In the search of a safe herbal contraceptive, Ocimum sanctum plant’s properties have been studied systematically in experimental animals. The long term feeding of fresh Tulsi leaves (465 mg/kg, /day) produced increase in body weight and decrease in weights of testes, prostate and adrenal gland. The fresh leaves intake led to changes like decrease in pH, hypotonic environment and chemical substances like mucoproteins, alkaline and acid phosphatase in spermatogenic cells leading to the formation of non-viable spermatozoa. However, male mouse could mate normally but no pregnancy occurred [524.] The benzene extract of Ocimum sanctum leaves at dosage of 100, 150 and 200 mg/kg, for 15 days altered the weight of testes significantly while it did not have any significant effect on epididymis, seminal vesicle, prostate and vas deferens. It was also observed to be effective in significantly reducing the sperm count and motility [525]. Long term (three months) feeding of Ocimum sanctum leaves (at dose of 20, 200 and 400 mg/100 g, body weight) was found to reduce sperm count, motility and weight of reproductive organs of male but weight remained unaffected in females. The mating behaviour of experimental rats reduced after 2 months of treatment but those female rats that mated, few carried full term pregnancy with normal gestation and delivered normal weighted offspring without any congenital defects. Hence Khanna et al suggested the possibility that Tulsi powder decreases testosterone levels directly or by inhibiting LHRH (leutinizing hormone releasing hormone) and preventing LH (leutinizing hormone) release necessary for bringing about mating response [526]. Significant decrease in sexual behaviour was observed in male rats at a doses of 200 and 400 mg/kg, p.o for 15 days. where the sexual behaviour in experimental rats were monitored by scoring different responses such as grooming, pursuit, mounting, intromission and ejaculation after giving graded doses [527].
11. Toxicity studies:

*Tulsi* is being used as medicinal herb for thousand years without any known adverse effects. There have been number of scientific studies conducted to evaluate the toxic effects of the plant. Bhargava and Singh [39] studied the toxicity to find out the lethal dose of ethanolic extract of *Tulsi* in adult mice. Approximate LD50 of *Ocimum sanctum* was found to be 4505±80 mg/kg body weight (bw) on administration by oral route and 3241±71 mg/kg, bw by intra-peritoneal (ip) routes. *Tulsi* leaves’ aqueous and alcoholic extracts were injected ip in mice with graded doses (3500–6300 mg/kg, bw) and mortality was observed for a period of 72 hours. The administration of aqueous extract did not produce any acute toxic symptoms (100% survival) at doses up to 5 g/kg, bw and the alcoholic extract was well tolerated (80% survival) up to a dose of 4g/kg, bw. The acute LD50 (30) values for aqueous and alcoholic extracts were found to be 6200 mg/ kg, bw and 4600 mg/kg, body weight respectively [518]. The toxicity of fixed oil (seed oil) of *Tulsi* has also been studied by intra-peritoneal administration in experimental rats. In acute toxicity study, fixed oil was given in a graded manner up to 55 ml/kg, bw but a dose of 30 ml/kg, bw were well tolerated. There was no mortality at 30 ml/kg, bw while 100% mortality observed at 55 ml/kg, bw. Whereas, sub-acute toxicity study, a dose of 3 ml/kg, bw was administered and no behavioral as well as histological changes were seen in the brain, lungs, liver and kidneys. The LD (50) of fixed oil was calculated and found to be 42.5 ml/kg, bw [528].