REVIEW OF LITERATURE
**STRESS AND STRESSORS**

Stress can be defined as the non-specific response of the body to any demand. Stress is reaction of the body to stimuli that disturbs its normal physiological equilibrium or homeostasis, often with detrimental effect\(^2\).

A stressor is a stimulus that causes an organism to deviate from the baseline functioning or homeostasis. A stressor will evoke behavioral and physiological responses from an organism in an attempt to survive and to regain equilibrium\(^2^8\).

Stressors can lead to suppression of immune function or to the onset of depression. The reaction of the body to stress depends on the novelty of stressor besides its quality and quantity. Stressors may be almost any disturbance – heat, and cold, environment poisons, heavy bleeding, poisons given by bacteria during infection, strong emotional reaction etc. Stressors differ among different people and even in the same person at different time. Important distinguishing characters of stress include its intensity and duration. The intensity of stress may be gauged by the peak levels of stress hormones, neurotransmitters and physiological changes such as increase in heart rate and blood pressure, and the duration of stress by the amount of time for which these changes persist, following the exposure to stressor.
SEYLE THEORY OF STRESS

Hans Seyle\textsuperscript{27} termed the body response to stressors as the general adaptation syndrome (GAS). The GAS consists of three stages, the alarm reaction, the stage of resistance and the stage of exhaustion.

The alarm reaction is essentially the emergency response of the body. In this stage, prompt response of the body, many of them mediated by the sympathetic nervous system, prepare us to cope with the stressors.

The stage of resistance of the stressor continues to be present. During this stage, certain hormonal responses of the body are important line of defense in resisting the effects of stressors. Especially important among the hormonal responses are increased activity in what is known as adrenocorticotrophic axis. Adrenocorticotrophic hormone (ACTH) is secreted into the blood stream by certain cells in the pituitary gland. The rate of ACTH secretion is controlled by corticotrophin releasing factor (CRF), released by certain cells in the hypothalamus. The CRF flows from the hypothalamus to the pituitary gland through hypothalamic hypophysial portal system. Stressors are able to activate the nerve cells or the hypothalamus so that more CRF is sent to the pituitary gland, thus increasing secretion of ACTH into the blood. Thus there is a major link between environmental event stressors and the bodily state of stress. ACTH stimulates cells in the outer layer of the cortex of the adrenal gland so that corticoid hormones, such as cortisol, are secreted into the
blood stream. Cortisol and other similar hormones, have many actions that allow the body to deal adaptively with stressors for long periods of time during the stage of resistance. Prolonged elevation of cortisol level can also have other harmful effects, such as raising blood pressure. In addition to cortisol there are other hormones, which in excess may have their own harmful actions, involved in the body response to stressors. The increased activity of the adrenal cortex in stress is not merely incidental but plays a genuine role in resistance as is shown by greatly increased susceptibility of adrenalectomised animals to stresses, and the protective fact by which administration of adrenal hormones can have normal animals under stress.

The final stage of the general adaption syndrome is the stage of exhaustion. In this stage, the body’s capacity to respond to those continuous and new stressors has been seriously compromised. For due instance, due to the actions of cortisol described above, a person may no longer be able to resist the infection and may become sick or die, or because of another stressor-induced hormonal effects, stomach ulcers, diabetes, skin disorders, asthma, increased susceptibility to cancer or a host of other diseases may occur at this stage or late in the stage of resistance\textsuperscript{27}.

The stress response uses the communication networks of the nervous and endocrine system to prepare the body to meet challenges such as imminent attack. The brain sends out chemical messengers in form of
stress hormones to almost every system and organ in the body. The result is increased heart rate and blood flow to the muscles, which enables the individual under attack to respond by either running or fighting. This phenomenon is better known as fight or flight response. The changes are beneficial in short term. But when the stress is chronic this short term benefits become long term liabilities\textsuperscript{28}.

**STRESS AND GLUCOCORTICOIDS\textsuperscript{29}**

In some circumstances chronic overreaction to stress can cause cell death. This could be because glucocorticoids inhibit neurons from using glucose, or by increasing the rate of calcium entry into cells. Calcium in large concentrations is toxic. In addition, glucocorticoids can decrease the expression of brain-derived neurotrophic factor (BDNF), which is necessary for cell proliferation in hippocampus. The brain is critical to interpret and respond to potentially stressful events; it is also the target for the actions of the stress hormones, particularly glucocorticoids. Therefore acute elevation of both catecholamines and glucocorticoids facilitate the formation of memories of events associated with strong emotions. Glucocorticoids (principally corticosterone in rodents) are released from the hypothalamic pituitary adrenal system during stress. Continued elevated circulating level of glucocorticoids can constitute a serious risk to organism. Thus following stressful situations it is important to terminate the release of glucocorticoids. The stress related
elevations of glucocorticoids level or accumulation of excitatory and inhibitory amino acids play a critical role in stress-induced dysfunctions. The stress-related elevations of glucocorticoids level alter the release or accumulation of excitatory amino acids, inhibitory amino acids and neuromodulators, which might cause cognitive impairment. However, there have been no studies to understand the changes or alterations in the level of major excitatory and inhibitory amino acids and its deleterious effects on the brain.

**STRESS AND THE BRAIN**

Stress has been implicated in many medical and psychiatric disorders. Although the acute stress response is adaptive, repeated, chronic, or single severe stress can initiate a long term neuronal changes that may underlie stress related pathophysiology. Integrating of the HPA stress response occurs by the way of interactions between stress-sensitive brain circuitary and neuroendocrine neuron of the hypothalamic paraventricular nucleus (PVN).

In the brain, the hippocampus is highly susceptible for various types of insults, which induces stress. Earlier studies have shown that chronic stress causes neuro-anatomical changes in the hippocampus, such atrophy or apical dendrites of CA3 pyramidal neurons, and also a reduction in corticosteroid receptors. Mild repeated stress induces the noradrenergic axon sprouting in cerebral cortex; these axons were from
the locus coerulus. It was suggested that sprouting might be an immediate adaption to stress. On the contrary, basolateral pyramidal neurons of the amygdale undergo hypertrophy. Alterations in the expression of neurotrophic factors in stress-induced hippocampal degeneration also occur.

**STRESS AND GASTROINTESTINAL SYSTEM** 31,32

Stress also induces gastric ulceration and chronic restraint stress-induced ulcerogenesis in the gastric mucosa may be brain driven event, because the hippocampal and entorhinal cortex lesions are known to aggravate ulcers is unclear, but their connection with the hypothalamic areas via relays in the amygdale may bring about this effect. Some studies suggest that the central amygdale modulates the degree of stress ulceration. The amygdale and the hippocampal formation apparently modulate the degree to which stressful experiences produce pathological changes in the gastrointestinal system.

**STRESS AND BEHAVIORAL PLASTICITY** 29

Adrenal stress and stressful experiences produce short term and reversible deficits in episodic and spatial memory in animal models and humans. However, repeated stress also impairs cognitive function in animals models and repeated glucocorticoid elevation or treatment in human is accompanied by cognitive dysfunction. Subsequent studies
showed that 21 days of repeated restrain stress impaired the short term (4 h) retention of spatial recognition memory in hippocampus–dependent Y-maze task. Recently, it was shown those 21 days of restrain stress results in an impaired acquisition and retention of spatial learning and memory. Furthermore, impairment in retrieval of long term spatial memory was directly related to the increase in circulating corticosterone levels at the time of testing. This suggests that there is direct increase in the adrenocorticoid levels and memory impairment. On the contrary, it has been demonstrated that suppression of the increased adrenocorticoid levels or the blockade of corticosterone synthesis with metyrapone blocks the stress induced learning impairment. Continous blockade of the brain glucocorticoids receptors or the treatment with tianeptine facilitates the spatial learning and memory in rats. In addition, systemic administration of corticosterone to non-stressed animals have shown dose-dependent impaired learning in spatial learning.

**EFFECT OF STRESS ON IMMUNE SYSTEM** 33-37

Cortisol and other similar hormones have many actions, which allow the body to deal adaptively with the stressors for long periods of time during the stage of resistance. Cortisol promotes the formation of glucose, a fuel needed for nerve and muscle activity, by breaking down fats and proteins. In the short run this is adaptive; the body has got more fuel. Proteins are needed for the manufacture of new cells. For
example, white blood corpuscles (WBC) which are critical for fighting infections, have a short lifetime and must be continuously replaced. If proteins needed to make new WBC are less, fewer WBC will be produced and the body will be less capable to fight infection. Moreover, cortisol has inhibitory action on the formation of antibodies. High levels of cortisol in the long run can seriously impair the body resistance to infection. Immunomodulatory agents of the plants and animal origin enhance the immune responsiveness against a pathogen by activating the immune system. Recent studies indicate the immunological changes occur during stress and experimental stressors modify the immune status of the organism. The central nervous system (CNS) being crucial for stress also regulates the immune function and studies show that common neural substrates like the hypothalamus are clearly involved in such CNS–immune system.

Neuropharmacological data have shown that neurochemical mechanisms regulate stress responses and transmitters like gama aminoburtyric acid (GABA) and endogenous opiates are crucially involved. There is an evidence indicating that CNS innervates lymphoid tissue. Lymphocytes bear receptors for several hormones and neurotransmitters, and pharmacological alterations in neural activity influence immunocompetence. Several reports show that GABA and endogenous opiates help to cope with stressful experience. Benzodiazepines, modulators of GABA, are known for their antistress
and antianxiety effects. Binding sites are reported on rat lymphocytes and opiate agonists and antagonist seemingly modulate some aspect of the immune function.

There is evidence of involvement of serotonergic system in establishing a relation between stress and humoral immune response, where an increase in 5-hydroxytryptamine (5-HT) levels in the brain, reduces humoral as well as cellular immunity.

Optimal support of the immune system is important for the prevention of disease, where acute illness or chronic degenerative disease due to microbial involvement or inflammation sets the stage for chronic disease, and for the initiation and progression of cancer. Immune system cells and the antibodies they produce attack any cell or molecule recognized as a foreign agent. The immune system is a complex system that involves specialized cells that communicate with each other via chemical messengers called cytokines.

**STRESS AND ADRENAL GLANDS**

Stress is known to cause adrenal hypertrophy and excessive glucocorticoid (GC) secretion or hypercortisolism which is paralleled by increased adrenal weights and reduced activities of the gonads. Glucocorticoids (GC) are adrenal steroids secreted by the adrenal cortex in response to stress and have a catabolic effect on metabolism, reproduction, growth, immune function and inflammatory response.
These actions can be viewed as crucial for adaptation to acute stress, as they result in more readily utilizable sources of energy and in the suppression of essential anabolic processes. Also, GC secretion is thought to counteract the effect of stress. Thus, GC, antagonize the CRH neuron and presumably the locus coeruleus–norepinephrine system and limit the stress response. However, chronic GC exposure whether as a result of prolonged stress or pathological GC hypersecretion can be profoundly deleterious. GC include natural hormones such as cortisol (hydrocortisone) in humans and other primates and corticosterone in rats, as well as synthetic analogues such as dexamethasone, prednisone and triamcinolone.

**MODELS USED FOR THE EVALUATION OF ANTISTRESS ACTIVITY AND PRINCIPLES INVOLVED**

**Swim Endurance Test**

Swimming in a restricted space from which the mice cannot escape and becomes immobile after the initial period of vigorous activity forms the basis of the last. It has been suggested that the observed immobility signifies behavioral despair resembling a state of mental depression and has been used to screen antidepressants. It is now recognized that this is a behavioral depression fairly common consequence of stress. It is also seen that the animal capability to cope with stress largely influences the
neurochemical consequence of stress. Thus exposure of animals to inescapable and severe stress leads to depletion of central noradrenaline and serotonin, postulated to be cause of endogenous depression.

**MODELS USED FOR EVALUATION OF ANTIOXIDANT ACTIVITY AND PRINCIPLE INVOLVED**

**Toxic Chemical-induced Liver Damage.**

Many toxic chemicals induce liver damage by inducing lipid peroxidation and/or oxidative damage to DNA. Assessment of antioxidant property is studied using liver homogenates, isolated liver cell membranes, DNA etc.

**Carbon tetrachloride Induced Hepatic Damage:**

Carbon tetrachloride is a potent hepatotoxic agent used to induce hepatic lesions, as well as evaluate the effects of drugs on liver. Short term administration of carbon tetrachloride causes hepatocellular injury with centrilobular necrosis and steatosis. Chronic administration may lead to liver cirrhosis (liver fibrosis) CCl₄ induces fatty liver and cell necrosis and play a significant role in triacylglycerol accumulation, increased lipid peroxidation, membrane damage, and depression of protein synthesis and loss of enzymatic activity. Carbon tetrachloride is converted into trichloromethyl radical (CCl₃) and trichloromethyl peroxy
radical (CCl$_3$ Ô$_2$). These radicals are extremely reactive and their duration of action is often short. Free radicals initiate lipid peroxidation of the biological membrane and bind covalently to lipids, proteins and nucleic acids. Lipid peroxidation leads to a cascade of events. These radicals are extremely reactive. CCl$_4$ induces the free radical formation and reduction in the antioxidant such as SOD and catalase in LTH. It is known that the biochemical markers are tissue specific and leak from the damaged tissue. Damage to the membrane induced by CCl$_4$ causes release of these enzymes in the serum and deficiency of enzymes in LTH. Administration of drugs showing antioxidant activity increases the level of enzymes in LTH and decrease in serum. The CCl$_4$ induces significant increase in the liver weight that is due to the blocking of secretion of hepatic triglycerides into the plasma.

**MODELS USED TO EVALUATE HEPATOPROTECTIVE ACTIVITY AND PRINCIPLES INVOLVED**

Carbon tetrachloride is a potent hepatotoxic agent used to induce hepatic lesions, as well as evaluate the effects of drugs on liver. When liver is damaged liver enzymes such as glutamate pyruvate (GPT), glutamate oxaloacetate (GOT) and alkaline phosphatase enter into the circulation. An increase in the level of these marker enzymes in the serum is an indication of liver damage.
Reduction in Prolongation of Pentobarbitone Sleeping Time

This method is used to screen carbon tetrachloride CCl₄ toxicity of drugs in animals. Hepatotoxic chemicals like CCl₄ reduce the level of drug metabolizing enzymes in the liver. Therefore metabolism of pentobarbitone is reduced resulting in the prolongation of pentobarbitone-induced sleeping time. If the plant drug reduces the CCl₄ induced prolongation of sleeping time, the drug can be considered as hepatoprotective against CCl₄ toxicity.

In the present thesis following drugs were used for the evaluation of antioxidant activity and adaptogenic activity.

Priyala sprang (Ayurvedic drug) Botanical name: Buchanania lanzan Spreng.

Vidakanachoornam (An Ayurvedic formulation)

Muppu (A Siddha drug)

DESCRIPTION OF PRIYALA AN AYURVEDIC PLANT DRUG:

**BUCHANANIA LANZAN**

Priyala is a drug used in the Ayurveda and the Unani system of medicine. It is known to have tonic, cardiotonic and astringent properties and is also used in the treatment of skin diseases. The gum from the bark is used for treating diarrhoea and intercostals pains and leaves are used for promoting wound healing. It is a commercially useful tropical plant. The plant is a medium sized evergreen deciduous tree, growing 50
ft tall. It bears fruits each containing a single seed, which is a popular edible nut, known as chironji. It is common in India mostly in eroded lands. It has tickly leathery leaves which are broadly oblong, with blunt tip and rounded base. Leaves have 10-20 pairs of straight parallel veins and are pubescent. All parts of the plant are used for the treatment of various disorders. The oil from the seeds is used to reduce granular swelling of the neck. Ointment made from the kernel is used to relieve itch and prickly heat.

PROFILE OF BUCHANANIA LANZAN

**Family:** Anarcardiaceae

**Kingdom:** Plantae

**Order:** Sapindales

**Genus:** Buchanania

**Species:** Buchanania lanzan

**Synonyms:** Buchanania latifolia, Chironjia sapida

**Sanskrit Names:**

Priyala, Piyala, Kharskandha, Bahulvalkala, Tapaseshtha, Sannakadru

Dhanushpat,

**Ayurvedic Properties**

Rasa: Tikta, Kashaya; Guna: Lakhu; Virya: Ushna
Medicinal Properties

Plant pacifies vitiated pitta, kapha,

**Plant parts used:** Bark, Fruit, Leaves

**Habitat:** *Buchanania lanzan*, which is cultivated across **India**, primarily in the northwest in dry areas.

**Plant Names in Different Languages**

**Common name:** Chironji Tree, almandette, calumpong nut, Cheronjee, Cuddapah almond, Hamilton mombin •**Hindi:** Char, chironji, chiraunji, piyal, pra-savak, priyal •**Marathi:** char, charoli, piyal •**Tamil:** charam • Malayalam: muungaappezh, nuramaram •**Telugu:** Char, charumamidi, priyaluvu, raj-adanamu •**Kannada:** Charoli, kole maavu •**Bengali:** Chironji, piyal, sarop •**Oriya:** charu, chanhra •**Konkani:** Char •**Urdu:** Chironji •**Assamese:** Piyal •**Gujarati:** Charoli •**Sanskrit:** akhatth, muni, piyala, prasavakh, priyala, rajanadanha, upavatth •**Nepali:** Chiraunjee.
Fig. 1. Tree of *Buchanania lanzan*

Chironji Tree (Fig.1.) is a medium-sized deciduous tree, growing to about 50 ft tall. It bears fruits each containing a single seed, which is popular as an edible nut, known as *chironji*. Fruits ripen from April to May and remain on the tree for quite a long time. It flowers from January to March. The plant avoids waterlogged areas, but occurs locally in clay soils. It can be identified by the dark grey bark with red blaze. It has tickly leathery leaves which are broadly oblong, with blunt tip and rounded base. Leaves have 10-20 pairs of straight, parallel veins, pyramidal panicles of greenish while flowers appear in early spring. Flowers are small, greenish white. In Chhattisgarh, it is common tree in dry deciduous forests. The Chironji fruits are considered as one of the
delicious wild fruits. The seeds are also eaten. The seeds are regarded as substitute for almonds. Chironji tree produces a gum similar to benzoin but inferior to Styrax benzoin.

Chironjani is an almost evergreen, moderate sized tree, with straight, cylindrical trunk, up to with 15 meters high and tomentose branches. Bark rough dark grey or black, fissured into prominent squares, 1.25-1.75 cm thick, reddish inside. Leaf thickly coriaceous, broadly oblong, obtuse, base rounded. Flower small, greenish-white, in axillary or terminal panicles. Calyx 3-5 lobed, 1 mm long, ovate, apex obtuse, petals 4-5, 3 mm long, ovate, sub acute. Disc fleshy, 5-lobed, stamens 10, inserted at the base of the disc, filaments linear. Ovary of 5-6 free carpels, situated inside the disc, only 1 carpel fertile. Drupel 8-12 in diameter. Seed hard stone, 4300-5300 / kg.

**Medicinal uses**

According to reference literatures related to different systems of medicine in India, roots, leaves, bark, fruit, seeds and gum are used medicinally. According to Ayurveda, it removes biliousness’, and cures blood disorders, fevers, thirst, ulcers, burning sensation of body. It is fattening, laxative, binding, cooling, aphrodisiac, cardiotonic, astringent to bowels, etc. According to Unani system of medicine, leaf juice is digestive, expectorant, aphrodisiac, purgative, blood purifier and delays thirst. Seeds are tonic to body and useful in treatment of gleet, urinary
concretions, fevers etc. The roots are acrid, astringent, cooling, depurative and constipating, and are useful in treatment of diarrhoea. Gum (stem exudate) is antidiarrhoeal and also used internally in rheumatism.

**Chemical Constituents**

Kernel lipids (65.6%), comprised mainly of neutral lipids (90.4%), consist mostly of triacylglycerol (82.2%), free fatty acids (7.8%) and small amount of diacylglycerols, monoacylglycerols and sterols. The leaves contain 2.64% tannins (0.35% gallo-tannins). The presence of triterpenoids, saponins, flavonoids and reducing sugars are also of reported. The presence of alkaloids, saponins and reducing sugars is also reported. The fatty acid composition of *Buchanania lanzan* seed oil, determined by urea complex formation and gas liquid chromatography, was found to be: myristic, 0.6; palmitic, 33.4; stearic, 6.3; oleic, 53.7; and linoleic, 6.0%. Triglyceride compositions of the native seed oil and its randomized product were calculated from the fatty acid compositions of the triglycerides and of the corresponding 2-monoglycerides produced by pancreatic lipase hydrolysis. The oil is composed of 3.2, 35.8, 45.5 and 15.5% trisaturated, monounsaturated disaturated, diunsaturated monosaturated and triunsaturated glycerides, respectively. The special characteristic of the *B. lanzan* seed oil is its content of 22.7, 31.0 and 11.3% dipalmitoolein, dioleopalmitin and triolein, respectively. The percent GS$_3$ content in the
oil increased from 3.2 to 705 by the process of randomization. On directed interesterification the oil yielded a product with a slip point of 41.5°C which may be suitable as a coating material for delayed action tablets. The oil also appears to be a promising one as a commercial source of palmitic and oleic acids.

**DESCRIPTION OF MUPPU (SIDDHA FORMULATION)**

The use of metals, minerals particularly mercury in medicine, which was a part of Tantric legacy, is seen in all ancient Indian medical school. This legacy is conspicuous in the so called Siddha System of medicine, which is now prevalent mostly in South India, especially in Tamil Nadu. This system dating back to pre-Ayurvedic period is of strong Tantric in orientation. It got mixed up with the cult of navakoti siddhas’ i.e., nine million siddhas, who transcended the death, preached a philosophy of transmuting the gross physical body composed of impure matter into the refined body of naturally pure matter, thereby making the body immutable and free from disabilities. In the present study one such mineral mixture Muppu extract was taken. Muppu is a combination of three kinds of salts prepared as per the palm leaf literature described in Kandarnadi Vaakiyam. These salts are processed from three different sources obtained in Tamil Nadu, India. The first salts is called vediuuppul/shivappu obtained from rocky salt formation in sea–shores of Trichundur district of Tamil Nadu. The third salt is procured by insertion
of bamboo sticks in deep sea at a location where three oceans meet. All the three salts are processed from the respective materials by extracting with amuri (specially prepared juice from banana tree) and purified as per procedures described in palm leaf literatures. Amuri is Tamil word and it denotes a sacred water like ambrosia, amirtha. In an unpublished manuscript called Kandar Nadi Vakkiyam, the term amuri is elaborately discussed. So far no scientific investigations are carried out on Muppu.

The samples were collected from Dr. Jai Prakash, Integrated Research Centre for Siddha Medicines, Bangalore. The hallmark of siddha medicine is Muppu. Muppu is said to be highly efficacious drug and kayakarpam is impossible without it. This is an alchemical preparation mentioned by all Siddha’s classical literature as combination of three salts which represent three elements of panchabootha as water, air and fire. The universal medicine of high order of quality is used in transmuting basar metals into gold and this is capable of curing radically all the diseases of the system. Muppu is also classified according to the preparations as vaidya Muppu, yoga Muppu, and vata Muppu. Present day physicians are using vaidya Muppu and is prepared as per the Kandar nadi vakkiyam.
DESCRIPTION OF VIDAKANACHOORNAM (AYURVEDIC FORMULATION)

The present study is aimed at the validation of the therapeutic efficacy of the traditional Ayurvedic preparation – Vidakanachoornam, used in Alappuzha district, Kerela, for liver disorders, steatosis (fatty liver) and as antioxidant. The constituents of the formulation are:

*Piper longum (Pippali); Moringa oleifera (Muringa); Embelia ribes (vidang)*

Each 100 g in quantity.