1. Preface

Living in today’s fast-paced world there are many stressors that we are faced with, these may include adapting to constant, rapid change; handling a major life event; juggling many roles and responsibilities; and going from one stressful situation to the next without taking time to relax. Learning to live and get ahead today is not possible without stress and this has become a fact of modern life. However, our physiological responses designed to cope with the ever-increasing adverse situations have not evolved appreciably during the past thousand years. The failure of successful adaptation during stressful situations has resulted in stress-related diseases that result from, or are associated with, dysregulation of the stress response (Chrousos and Gold, 1992). Stress isn’t always bad. In small doses, it can help to perform under pressure and motivate you to do your best. But when you’re constantly running in emergency mode, your mind and body pay the price, stress becomes distress and can interfere with functioning effectively. Chronic distress increases the risk for many health disorders, including coronary heart disease, hypertension, eating disorders, ulcers, diabetes, asthma, depression, migraine headaches, sleep disorders, chronic fatigue, and certain types of cancers. The term "stress" was first used by psychologists before the endocrinologist Hans Selye in the 1930s. He later broadened and popularized the concept to include the response of the body to any demand. In Selye's terminology, "stress" refers to a condition, and "stressor" to the internal reaction causing stress. In context of our lives, stress is generally taken to refer to a situation that result in a challenge or threat to our adaptive capacity – something that disturbs our dynamic equilibrium or homeostatic balance. In other words, it is perception or cognitive assessment made by an individual about the impact of particular circumstance or constellation of events on his or her life. When we sense danger – whether it’s real or imagined – the body's defenses kick into high gear in a rapid, automatic process known as the “fight-or-flight” reaction, or the stress response. The stress response is the body’s way of protecting us. When working properly, it helps us to stay focused, energetic, and alert. But beyond a certain point, stress stops being helpful and starts causing major damage to our health, our productivity, and our quality of life. Stress adaptation systems include the hypothalamus-pituitary-adrenal (HPA) axis (Dowing JG et al., 2000; Korte SM et.al., 2005; Wrona D et al., 2006), the sympathetic nervous
system (Sanders VM et al., 2006.) and the parasympathetic nervous system (Deriaz O et al., 1989). An acute stress response immediately involves the release of catecholamines from sympathetic neurons and, within minutes, the stimulation of the HPA axis with an elevation of glucocorticoids in the circulation (Korte SM et al., 2005, Wrona D et al., 2006). In addition, increased catabolism, increased breathing rate and heart rate, and increased muscle tone contribute to the classic fight-or-flight response (Korte SM et al., 2005; McEwen BS et al., 2004). The acute stress response is normally short lived and resolves within minutes or hours or adapts to the new environmental conditions. In contrast, when stress becomes chronic, the HPA axis and other neuroendocrine pathways may be activated continuously and cause prolonged activation of enhanced catabolism leading to exhaustion of the body’s reserves (Korte SM, et al., 2005; Dhabhar FS et al., 2001; McEwen BS et al., 2004.) In addition, chronic stress may cause behavioral changes, including depression, increased anxiety, fatigue and memory loss (Quervain DJF et al., 1998; Strekalova T et al., 2005). Finally, an elevated level of glucocorticoids is immunosuppressive, increasing the risk of infection, and slowing wound healing (Korte SM et al., 2005; Dhabhar FS et al., 2001; Eskandari Fe et al., 2002).

1.1. Stages of Stress:
In response to stressful events, one can experience one, two or all of the following stages:

*Stage 1: Mobilization of Energy*

All bodily activity is increased in response to a stressor that is frightening, such as a near car accident. This starts the body's ‘fight or flight' reaction, causing the release of adrenalin. One feels the heart pounding and palms feeling sweaty. This is called primary stress.

*Symptoms:*
- increased heart rate and blood pressure
- rapid breathing
- sweating
- decreased digestion rate, creating butterflies and indigestion.
Stage 2: Exhaustion or Consuming Energy

If there is no escape from Stage 1, the body will begin to release stored sugars and fats, using up its bodily resources.

*Symptoms:*
- feeling driven
- tiredness and fatigue, an increase in smoking, coffee drinking and/or alcohol.
- anxiety
- memory loss
- acute illnesses such as colds and flu.

Stage 3: Draining Energy Stores

If the stressful situation is not resolved, you may become chronically stressed. The body's need for energy resources exceeds its ability to produce them.

*Symptoms:*
Serious illnesses such as:
- heart disease
- ulcers
- mental illness
As well as:
- insomnia (difficulty in sleeping)
- errors in judgement
- personality changes
and above all Immunosuppression.

1.2. Stress -immune relation.

Interplay between the immune, nervous and endocrine systems is most commonly associated with the pronounced effects of stress on immunity and leaves the host vulnerable to opportunistic diseases. Immune system plays a very important role defending the body against variety of infectious diseases and any abnormality or change in its ability to perform normally can have greater implications for health. It is inherently dynamic system wherein various organs, tissues, cells and cell products like cytokines,
immunoglobulins and other mediators work together in defending the body against a variety of infectious diseases. Any abnormality or change in its ability to perform normally can have greater implications for health. Sympathetic fibers descend from the brain into both primary (bone marrow and thymus) and secondary (spleen and lymph nodes) lymphoid tissues (Felten & Felten, 1994). These fibers can release a wide variety of substances that influence immune responses by binding to receptors on white blood cells (Ader, Cohen, & Felten, 1995; Felten & Felten, 1994; Kemeny, Solomon, Morley, & Herbert, 1992; Rabin, 1999). Second, the hypothalamic–pituitary–adrenal axis, the sympathetic–endocrine axis, and the hypothalamic–pituitary–ovarian axis secrete the adrenal hormones epinephrine, norepinephrine, and cortisol; the pituitary hormones prolactin and growth hormone; and the brain peptides melatonin, -endorphin, and enkephalin. These substances bind to specific receptors on white blood cells and have diverse regulatory effects on their distribution and function (Ader, Felten, & Cohen, 2001). Moreover, people’s efforts to manage the demands of stressful experience sometimes lead them to engage in behaviors—such as alcohol use or changes in sleeping patterns—that also could modify immune system processes (Kiecolt-Glaser & Glaser, 1988). Thus, behavior represents a potentially important pathway linking stress with the immune system. Both major and minor stressful events can have direct adverse effects on a variety of immunological mechanisms; both animal and human studies have provided convincing evidence that these immune alterations are consequential for health. Restoration of altered immune system involves coordinated interaction of various signaling molecules with several immune cells and facilitation of the cross-talk between these immune cells to evoke a desired immune response. T lymphocytes play a major role in immunity and are sub divided in to Th1 and Th2 helper cells on the basis of their cytokine secretion that eventually determine the induction of cellular and or humoral immune responses. The appropriate choice of Th1 or Th2 cytokine profile is crucial and the dichotomy of this selection is regulated early in the course of the immune response. Hence, the optimal immunotherapy should restore or maintain a well balanced Th1 and Th2 response, suited to the immune challenge (Khan et al., 2010).
1.3. Herbal medicine- Natural remedy for stress ailments.

Though there is a long list of drugs to handle stress, the list of their side effects like headaches, back ache, neck pain, frozen shoulders, and other ailments is equally long. So everyone is on the lookout for natural stress relief techniques which are very effective and at the same time don't have many side effects. Plants have been the basis of treatment of human diseases since time immemorial. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics, because plants have given to mankind, a large variety of drugs, which could not be replaced by synthetics even today (Labadie RP, 1993).

A large number of herbal drugs are mentioned in Ayurveda, for their immunomodulating activity, adaptogenic and rejuvenating properties (Nayer RC, 1979; and Labadie RP, 1993). Pharmacological agents that induce a state of non-specific increase of resistance of the organism (SNIR) to aversive stimuli that threaten to perturb internal homeostasis are known as adaptogens (Brekhman, 1965; Brekhman and Dardymov, 1969; Voskresnsky et al., 1986). Since the introduction of adaptogens several plants have been investigated, which were once used as tonics due to their adaptogenic and rejuvenating properties in traditional medicine (Rege NN et al., 1999). These herbal stress busters help us to combat the negative effects of stress and improve resistance thus improving our health and well-being. They increase the body’s vitality, enhance the life-force, encourage natural harmony and generate radiant health. A primary adaptogen/ herbal antistress agent are a remedy which: 1) Must be safe and have no side effects, even with prolonged consumption. 2) Must enhance the general resistance of the entire body. 3) Must act in a non-specific way having a ‘normalizing effect’ against all forms of stress. 4) Must have an ability to maintain or restore homeostasis. 5) Must also have solid scientific research validating its use as a stress busting agent.


More recent research postulates that antistress agents work primarily by affecting the Hypothalamic-Pituitary-Adrenal (HPA) axis and the Sympathoadrenal System (SAS) (Panossian, A., 2003). Thus, antistress agents modulate our response to stress (physical, environmental, or emotional) and help regulate the interconnected endocrine, immune,
and nervous systems. This re-regulation of a disordered or highly stressed system is achieved by metabolic regulators such as cytokines, catecholamines, glucocorticoids, cortisol, serotonin, nitric oxide (NO), corticotrophin-releasing factor (CRF), and sex hormones. When under stress, antistress agents help the adrenal glands to mount an immediate hormonal response, by manufacturing and releasing more stress hormones such as cortisol. But when stress stops, they help the adrenal glands to shut down more quickly. If stress is prolonged and severe, the adrenal glands reserve their resources by reducing the amount of hormones released due to adaptogenic restoration of hypothalamic receptor sensitivity. This conserved energy is available to continue the body’s responses to stressors, thereby delaying adrenal exhaustion. The biological machinery that handles energy in the body is stimulated by antistress agents. Consequently, during challenge or stress, more sugar is released into the blood from the body’s store houses. This glucose is quickly taken up by the tissues to carry out their work. Stress busters help glucose to cross the cellular membranes more easily. In the blood, the levels of sugar return more quickly to normal. Thus, antistress agents 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. They help us to cope with stress more effectively, psychologically, mentally and emotionally. They enable us to stay strong and healthy during times that we may otherwise get sick. They also resist or delay many of the negative effects of aging by providing us with better physical, mental and sexual energy; they delay the effects of aging of the eye, skin, heart, and all the organ systems and improve sleep that might otherwise be disrupted. It is tempting to consider that the restorative and rejuvenating power of these drugs is largely due to their action on immune system, the main defense system of the body. Any alteration or change in the normal functioning of this defense mechanism can lead to a wide variety of health hazards. Stress causes physiological changes that tend to weaken our immune system. When immune system becomes compromised, our health can be negatively affected: infections and illness occur more frequently, and a variety of immune system disorders take place. In chronic stress conditions, hypothalamic-pituitary-adrenal (HPA) axis activation-induced immunosuppression is associated with increased concentration of circulating
corticosterone and impaired Th1/Th2 balance. The antistress agents have the ability to restore the altered immune function by activating macrophages, natural killer cells, antigen-dependent T lymphocytes; regulate cytokines profile thereby, providing an ability to suppress tumor growth, enhance tissue differentiation, improve intercellular adhesion, and reduce the likelihood of metastasis. Therefore, herbal stress busters hold great promise for the development and prevention of chronic stress related illness due to their ability to enhance our resistance to a variety of adverse influences with lesser side effects.

2. Aim of the present study.
The present study was undertaken to explore a stress busting agent of herbal origin having potential to restore stress induced immunological, biochemical and behavioural alterations.

Supportive aims:

➢ To study the effect of plant extract(s)/fraction(s) on various immune parameters in normal and immune suppressed animals.
➢ To study the antistress/ adaptogenic potential of active plant extract(s)/fraction(s) on various stress induced immunological, behavioral and biochemical alterations.
➢ To study the different molecular mechanisms of the active moieties to understand their possible therapeutic potential as anti stress agent(s).
➢ To achieve and maintain control of stress symptoms with the help of plant extract(s)/fraction(s) through modulation of immune responses.
➢ Focus on the studies with novel approaches for evaluating different stress associated immune parameters.

3. Plants taken up for the present study.
Two plants, *Taraxacum officinale* and *Cicer microphyllum*, were selected for the study on the basis of their traditional medicinal uses.

3.1 *Taraxacum officinale*.

3.1.2 Description:
*Taraxacum officinale* (TO) (dandelion), a member of the Asteraceae family, grows to a height of about 12 inches, producing spatula-like leaves and yellow flowers that bloom
year-round. Upon maturation, the flower turns into the characteristic seed-containing puffball. Common Dandelion originated from Eurasia and now is naturalized throughout North America, southern Africa, South America, New Zealand, Australia, and India.

**Fig.1.2 Taraxacum officinale.**

### 3.1.3. Medicinal uses:

This plant has long been used as medicinal herb round the globe. The first evidence for its therapeutic use was mentioned by Arabian physicians of the 10th and 11th centuries to treat liver and spleen ailments (Sweeney et al., 2005). Especially the utilization of dandelion in liver complaints was largely based on the Doctrine of Signatures. This has long been used in folk medicine to treat hepatic disorders and some women’s diseases, such as breast and uterus cancers, and as lactating, choleretic, diuretic, and anti-inflammatory remedies (Ahmad et al., 2000; Kisiel and Barszcz, 2000). This plant was also reported to have antiangiogenic, anti-inflammatory and anti-nociceptive activities (Jeon HJ et al., 2008). It is also considered to be a “blood purifier” and is employed as a mild laxative, for treating arthritic and rheumatic complaints as well as eczema and other skin conditions in popular medicine (Bisset et al., 1994). Decoction of the whole plant is traditionally used in Mexico to control Diabetes mellitus (Hernandez-Galicia et al., 2002). In Turkish popular medicine, the herb is applied as a laxative, diuretic and potent anti-diabetic medicine (Önal et al., 2005 and Ertaş et al., 2005). The Traditional Chinese Medicine knows dandelion, sometimes in combination with other herbs, to treat hepatitis, to enhance immune response to upper respiratory tract infections, bronchitis or pneumonia, and as a compress for its anti-mastopathy activity (Leu et al., 2005 and Sweeney et al., 2005).

Although TO is a well-known traditional herbal remedy with a long history, until recently only limited scientific information is available to justify the reputed uses.
3.2. *Cicer microphyllum*:

3.2.1. Plant description:

*Cicer microphyllum* Benth (synonym- *Cicer soongaricum*) belongs to family leguminosae or Fabaceae. Locally it is known as Kukunnory, Seri or Surri and wild gram in English. It is a herbaceous plant, growing up to 0.15m and is widely distributed in western Himalayas from Afghanistan to Tibet and western Nepal. This plant is a wild relative of cultivated chickpea and is a high altitude cold adapted species.

![Fig: 1.3 Cicer microphyllum](image)

3.2.2. Traditional medicinal uses:

The leaves of the plant are used locally to cure bronchitis and as astringent to bowels. The unripened seeds are considered as stimulant, tonic, and aphrodisiac. The seeds are also used to cure thirst and burning sensation of stomach and has also been claimed to be used as anti-helminthic (Gorsi M.S. et al., 2002). Whole plant is used for increasing milk production and as general tonic for cows (Basant B. et al; 2007). But this plant is still an unexplored wealth of Himalayan terrain as no scientific data proving its traditional uses has been reported so far.

Since both of these plants have wide traditional usage in the treatment of variety of diseases, it was hypothesized that their claimed restorative effects are chiefly due to their effect on immune parameter as the immune system plays a very imperative role in defending the body against the diverse ailments. Stress and depression are one of the most common factors leading to disturbed immunestatis. It increases the risk of adverse health outcomes by suppressing the immune response in a fashion that leaves the host...
susceptible to opportunistic infections. Therefore, we carried out a detailed investigation of immunomodulatory potential of these plants’ extracts /fractions in normal and then in stress induced immune-compromised conditions to see the restorative effect of these herbal drugs on the immune profile of chronically stressed animals employing well established animal models.