Preface

Stress is defined as the non-specific response of the body to any demand. It is an alarm process that warns about disrupted homeostasis and helps to restore it by inducing the “fight or flight” response (Selye, 1936, 1956). As such, stress accounts for nearly everything disturbing the daily routine of an individual and challenging to adapt to changes (Selye, 1936). Since the pioneering research of Hans Selye (Selye, 1936), it has been accepted that stress has a number of effects on physiology and behavior, some beneficial and some aversive. Stress can provide organisms with the energy to act rapidly in threatening situations or stress can be overpowering, debilitating and lead to both physiological and behavioral impairments. Early work discussed stress as an elicitor of the well known fight-or-flight response, which leads to an increased release of neurotransmitters (e.g., epinephrine and norepinephrine) and stress hormones (e.g., cortisol) throughout the body, which helps prepare an animal for action. Hence, a brief period of controllable stress is rather beneficial to health. In contrast, lack of control and uncertainty can produce a chronic state of hyper activation of the stress axis where the stress mediators are not turned off adequately. In case of an inadequate or extremely prolonged stress response, where the allostatic systems remain active over a long period of time, the costs of reinstating homeostasis might be too high. This condition is termed allostatic load or overload (McEwen and Stellar, 1993; Schulkin et al., 1994; McEwen, 1997) and is generally considered to enhance vulnerability to disease. Allostatic load leads for instance to impaired immunity, atherosclerosis, obesity, bone demineralization and atrophy of nerve cells in the brain. Many of these processes are seen in major depression and in anxiety disorders (McEwen, 2002).
Stress hormones are known to cause alterations in metabolism in addition to changes in other physiological processes. It is a well-known fact that chronic stress induces dyslipidemia by altering the lipid metabolism. There are many reports which reveal the stress induced alterations in the lipid profile (Berger et al., 1980, Bryant et al., 1988, Ruiz de Gordo et al., 1994, Ricart-Jane et al., 2002, Li et al., 2003, Sato et al., 2006, Akhtar et al., 2008, Shanmugapriya et al., 2012). These studies involved a short duration of stress exposure. In addition, it is not known whether or not, the effects of chronic stress on blood lipid profile are duration of exposure dependent and reversible. The 1st chapter addresses these lacunae in our understanding of stress and lipid metabolism.

On the other hand, acute and chronic stress also affects the antioxidant status. There are many reports which reveal the alterations in the antioxidant enzyme activities and concentrations of antioxidants which lead to oxidative damage (Liu et al., 1994, Kovacs et al., 1996, Oishi and Machida, 2002, Torres et al., 2004, Zaidi et al., 2005, Ates et al., 2006, Bhat et al., 2007, Zafir and Banu, 2009). Despite these studies, the effect of long term exposure and whether the effects are duration dependent or not are not known. In addition it is also not known whether prolonged chronic stress induced oxidative damage returns to normalcy after cessation of stress exposure or requires a long duration to reach normalcy or irreversible.

It is evident from the above description that stress induces alterations in blood lipid profile on one hand and antioxidant status on other hand. These two factors are mainly responsible for the development of atherosclerosis. There are a few reports on stress coupled with fat diet induces the atherosclerotic development in the experimental animals (Shively et al., 1989, Bernberg et al., 2008, Neves et al., 2009).
However, it is not known, whether chronic stress alone despite normal diet leads to atherosclerotic development or not. This question is also addressed in the first chapter.

Since, metabolic disorders and sufferings due to chronic stress are mediated by glucocorticoids, methods to alleviate negative effects of stress have to be more focused on antagonizing the deleterious effects of glucocorticoids. However use of glucocorticoid antagonists for health purpose may not be feasible because of high cost and possible negative side effects. On the other hand suppressing HPA axis activation under stress is another approach. Moreover there are no studies utilizing antagonists of HPA axis to suppress the stress induced alterations in the metabolism and antioxidant status. Hence there is a dire need to investigate the efficacy of naturally occurring compounds of plant origin which are used in traditional medicine for their anti-stress effects. The advantage of such compounds is that they are time tested compounds without side effects.

Indian system of medicine (Ayurveda) has several herbal preparations which are known to possess anti-stress effects. However, scientific validation using scientific methodology is needed. Whether these herbs suppress the elevated HPA axis under stressful condition and prevent stress induced alteration in metabolism and antioxidant status are not investigated. One such herb, which is known to possess a variety of medicinal properties, is *Withania somnifera* (Dunal), commonly called Indian ginseng or ashwagandha. Earlier studies have reported the multiple therapeutic properties of ashwagandha such as antioxidant, adaptogenic, aphrodisiac, liver tonic, astringent, anti-inflammatory and antiulcer agent (Gupta and Rana, 2007; Bhatnagar, 2009).

There are a few reports on the effect of ashwagandha on stress induced alterations in antioxidant status but the doses are high. Further, although *W. somnifera*
is known to have several protective effects, specifically against stress, thus far there are no experimental studies to demonstrate simultaneous suppressive effects of *W. somnifera* on stress induced dyslipidemia and oxidative damage at a suitable dose. This is an important aspect to be investigated as these two are major risk factors for atherosclerosis. Studies on these lines will reveal efficacy of *W. somnifera* in preventing stress induced alterations, which is yet to be investigated. Hence in the present study different organic solvent extracts of ashwagandha have been investigated for their suppressive action on alterations in corticosterone secretion, lipid metabolism and antioxidant status.

`The thesis has been divided into 2 chapters. The first chapter deals with the detailed investigation on the effects of acute and chronic stress on antioxidant status and alterations in lipid profile and reversibility of effects. The second chapter deals with extraction of ashwagandha roots with different solvents and testing the extracts for antioxidant (*in vitro*) property. The most potent extracts are further investigated for their lipid lowering and antioxidant actions under chronic stress. Ethanolic and chloroform extracts were effective in preventing the chronic stress induced alterations in lipid profile and antioxidant status. The novel finding was that both ethanolic and chloroform extracts prevented the chronic stress induced oxidative damage and dyslipidemia probably by suppressing the activated HPA axis.

In each chapter, the lacunae existing in the field and the need for the present investigation have been highlighted citing the earlier work under the section ‘Introduction’. The description in the introduction finally leads to the statement of objectives. The experimental protocols and methodology have been described to achieve the proposed objectives under “Materials and methods”. The results of
different experiments in each chapter have been presented under the section “Results” supported by tables and figures (graphical representations). The results are interpreted and the significance of the results of the present study is highlighted citing investigations made by earlier workers under the section “Discussion”. At the end of each chapter a “Summary” of the work describing briefly objectives, methods, results and conclusions has been provided. The earlier work referred in the text has been listed under “References” with all the bibliographic information at the end of the thesis.