CHAPTER – 1

INTRODUCTION

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Roots of all systems of medicine go back to ancient medical herbalism. The classical texts of one of the oldest traditionally known Indian system of medicine; i.e. Ayurveda, are the oldest known ones recommending medicinal uses of numerous herbs together with appropriate healthcare practices and food habits for prevention of health problems, and cure of diseases. They are also the oldest known ones recognizing the fact that due attention has to be paid to mental health problems for prevention and cure of diseases, illnesses and their symptoms. These and many other Ayurvedic therapeutic principles have now been well accepted by almost all currently practiced traditional systems of medicine, as well as by most scholars, practitioners and healthcare authorities around the globe. Despite such global acceptance, mental health problems still continue to be the major health burdens of all modern societies [M. Prince et al., 2007]. This is mainly because currently available and commonly recommended therapeutic modalities, psychoactive and other drugs, phytopharmaceuticals, nutraceuticals, and diverse other healthcare products do not appropriately meet the therapeutic demands of a vast majority of the global population. Therefore, extensive efforts are now being made in many laboratories to identify novel therapeutic leads potentially useful for properly meeting the mental health challenges of the 21st century.

Diverse modernized and globalised versions of Ayurvedic system of medicine and healthcare are still widely practiced in India and elsewhere, and popularity of diverse therapeutic modalities mentioned in ancient Ayurvedic texts has continued to increase during more recent decades [D. Wujastyk and F.M. Smith, 2013]. However, during the course of the history, nomenclatures of plants and other natural products of medicinal value mentioned in ancient Ayurvedic texts have undergone a variety of changes. Such has been the case also for the medicinal plant called ‘Ashwagandha’ in Sanskrit, i.e. the language used in classical

Ayurvedic texts, and many other modern Indian languages. In Ayurvedic system of medicine, Rasayana therapies are recommended for almost all metabolic and stress associated medical conditions. Amongst all Rasayana herbs and their combinations widely used or recommended by modern Ayurvedic practitioners, *Withania somnifera* is pre-clinically as well as clinically one of the most well studied one. According to modern Ayurvedic scholars, *Withania somnifera* is the botanical name of the plant Ashwagandha, diverse medicinal uses of different parts of which have been known since long in other traditionally known systems of medicine in India and other countries [Q. Uddin et al., 2012]. Although it is now often cultivated in India, Pakistan, Nepal and China for medicinal purposes, diverse medicinal uses of different types of concoctions obtained from different parts of the plant (growing wildly) are also not very uncommon.

In classical Ayurvedic texts, Ashwagandha is considered to be an important rejuvenating herb, and its roots are often used as one of the major ingredients of many traditionally known Ayurvedic formulations currently commercialized in India and other countries for promoting longevity, energizing the body, attenuate the aging process, and augmenting defence against infectious diseases, or as tonics [G.L. Gupta and A.C. Rana, 2007; K. Narinderpal et al., 2013; R. Tiwari et al., 2014]. The major medical conditions now commonly treated with *Withania somnifera* extracts are anxiety, depression, memory impairment, emotional stress, male impotence, and other metabolic disorders like diabetes, obesity and gout [S.K. Bhattacharya and A.V. Muruganandam, 2003; S.K. Bhattacharya et al., 2000; G.L. Gupta and A.C. Rana, 2007; K. Narinderpal et al., 2013]. Information now available on pharmacology and medicinal phytochemistry of various types of *Withania somnifera* extracts obtained from different parts and sources of the plant have often been reviewed during more recent years [V. Kumar et al., 2015a]. However, many questions concerning its bioactive constituents, their modes of actions, and appropriate cultivation and extraction procedures necessary for

obtaining diverse medicinal benefits from the plant still remain unanswered, or at the best remain speculative only. This is mainly because the contents of bioactive secondary metabolites of the plant vary considerably in different parts of the plant cultivated and harvested under different conditions, and depending on the processing and extraction procedures used their contents considerably vary also in different types of extracts obtained from a given part of the plant [R.S. Sangwan et al., 2004; N.S. Sangwan and R.S. Sangwan, 2014]. Such are the cases not only for *Withania somnifera*, but also for numerous other medicinal plants now well recognized as adaptogenic herbs and traditionally used for stress relief or for improving strength, stamina, and longevity [D. Winston and S. Maimes, 2007].

The steroidal lactones commonly known as withanolides were the very first bioactive secondary metabolites of the plant identified [E. Glotter, 1991; S.K. Kulkarni and A. Dhir, 2008], and most currently commercialised *Withania somnifera* extracts are now often analytically characterised by, or standardised on, their contents of total withanolides [M.I. Choudhary et al., 2013; L.C. Mishra and B.B. Singh, 2000; M. Elsakka et al., 1990]. Results of numerous preclinical and some clinical studies now available on them strongly suggest that *Withania somnifera* extracts containing sufficient quantities of withanolides could also be used as therapeutic alternatives for treatments of various inflammatory disorders including cancer and arthritis [M. Kaileh et al., 2007; R.R. Kulkarni et al., 1992; S.A. Ghawte et al., 2014]. However, apart from withanolides diverse other bioactive secondary plant metabolites with reported anti-stress, anxiolytic, anti-inflammatory and other therapeutically interesting bioactivities in animal models are also encountered in such extracts. During the past decade, several reports revealing anxiolytic, antidepressant, and stress response suppressing or adaptogenic activities of diverse types of *Withania somnifera* extracts containing withanolides, or devoid of them, have appeared also [N.J. Dar et al., 2015; M.A. Pratte et al., 2014; R. Wadhwa et al., 2016]. The observations reported in clinical studies dealing with the

effects of *Withania somnifera* roots extract on stress and anxiety have revealed that, the extract caused significant improvement in reducing stress and anxiety in human volunteers as compared to the placebo group [K. Chandrasekhar et al., 2012; U. Pingali et al., 2014]. In similar other studies, standardized roots extract of *Withania somnifera* improved the cognitive capacity in bipolar disorder, muscle strength and stamina, metabolic conditions like hyperglycemia or hyperlipidemia as compared with those observed in subjects not undergoing any other additional treatments [S. Wankhede et al., 2015; K.N. Chengappa et al., 2013; B. Andallu and B. Radhika, 2000; A.A. Raut et al., 2012]. In another study, a highly concentrated Ashwagandha root extract caused significant improvement in sexual function in healthy female volunteers as compared to the placebo group [S. Dongre et al., 2015]. Available reports on clinical studies with *Withania somnifera* extracts have continued to add experimental evidences in favor of the convictions of the researchers and Ayurvedic practitioners that the plant could also be used for the prevention of mental health problems and metabolic disorders and also for the improvement of the physical strength and stamina.

In some other studies, it has been reported also that diverse bioactive plant phenolics, flavonoids, and many other phytochemicals are also encountered in *Withania somnifera* [R. Wadhwa et al., 2013; S. Chatterjee et al., 2010; A. Bhatia et al. 2013; S.K. Bharti et al. 2011].

Historically, the concept that medicinal plants traditionally often used as tonics or rejuvenators can be pharmacologically classified as herbal adaptogens evolved from the experimental observation made in Russia using *Panax ginseng* and diverse other medicinal plants growing in China and Russia [II. Brekhman and IV. Dardymov, 1969]. During 1988, a review pointing out pharmacological similarities between *Ashwagandha* and Ginseng was published [B. Patwardhan et al., 1988], and one of the very first reports comparing the anti-stress effectiveness of the roots of the two traditionally known medicinal plants appeared during 1994 [A. Grandhi et al., 1994]. Since then, numerous others revealing

pharmacological similarities between *Withania somnifera* (family Solanaceae; genus Withania) and *Panax ginseng* (family: Araliaceae) have continued to appear. Therefore, many modern herbal researchers as well as modern Ayurvedic scholars and practitioners now often consider *Ashwagandha* to be "Indian Ginseng" [S.K. Kulkarni and A. Dhir, 2008; R. Seenivasagam et al., 2011]. However, several decades of extensive efforts made to identify therapy relevant bioactive constituents of *Panax ginseng* itself have not yet been able to define the pharmacological and pharmaceutical qualities of its extracts necessary for obtaining consistent medicinal benefits from them [V. Vuksan and J.L. Sievenpiper, 2005; H.D. Yuan et al., 2012; R. Ramaswami and J. Stebbing, 2013]. Analogous is the situation also for *Withania somnifera* and all other better-scrutinized Ayurvedic herbs now often used also for treatments of diverse medical conditions resulting from health risk factors other than those already known before the 20th century. On the other hand, despite widespread medicinal uses of such traditionally known herbs and modern drugs, health burden of numerous communicable as well as non-communicable diseases have continued to increase during more recent decades [G.M. Husain et al., 2011a; A.K. Thakur et al., 2013a; 2013b; 2014a; 2014b; 2014c].

In view of the situation, attempts are now being made in several laboratories to follow the so-called "reverse pharmacology" strategy for identifying drug leads from traditionally known medicinal plants, or for defining of their diverse therapeutically interesting pharmacological activity profiles [B. Patwardhan et al., 2008; M.L. Willcox et al., 2011; B. Graz et al., 2012]. However, during most such efforts little attention is paid to the fact that the ultimate fate of such efforts depends also on the relative concentrations of diverse bioactive secondary plant metabolites present in the starting plant material and processing procedures used for preparing the formulations used in clinical and other studies necessary for initiating such projects [K.K. Gupta et al., 2010]. Since in Ayurvedic and other traditionally known medical

systems diverse types of formulations from different parts of *Withania somnifera* are used in combinations with other herbs and natural products, experimental observations made with a given type of extract of the plant must not necessarily be very reliable predictors of diverse traditionally known medicinal values of the plant. Complexities of the problems arising from such facts are now well recognized, and several alternative strategies for resolving them have been proposed also [I. Raskin and C. Ripoll, 2004; A. Agarwal et al., 2014; T. Efferth and E. Koch, 2011; J. Gertsch, 2011]. However, most such proposed or currently practiced strategies are based on reductionist pharmacological concepts of modern medicine, and often neglect the holistic therapeutic principles of Ayurvedic and most other traditionally known systems of medicine and healthcare. Moreover, diverse modern technologies and laboratory facilities necessary for implementing such strategies are not yet easily accessible and affordable to numerous herbal researchers interested in obtaining therapeutic leads from traditionally known medicinal plants, or involved in efforts necessary for translating holistic therapeutic principle of Ayurvedic and other traditionally known systems of medicine in terms of modern medical sciences.

Since diverse traditionally known medicinal uses of *Withania somnifera* can now be better explained, by its adaptogenic properties, attempts are now being made to identify diverse bioactive secondary metabolites of the plant potentially involved in such efficacy of its diverse types of extracts. More recently, it has been reported, indeed, that water soluble molecules structurally unrelated to withanolides also possess therapeutically interesting bioactivities [R. Wadhwa et al., 2013], and that the contents of known or unknown bioactive constituents of different parts of the plant harvested and processed under different conditions vary enormously and can have therapeutic potentials other than those known traditionally [R. Wadhwa et al., 2016]. Since withanolides and diverse other bioactive secondary metabolites of the plant also encountered in the aerial parts (leaves with, stems, and fruits etc.) of the

plant [S. Chatterjee et al., 2010; R.S. Sangwan et al., 2004], it now seems reasonable assumption that more easily harvestable parts of the plant could also be used as substitutes of its roots still often used for formulating and commercializing Ayurvedic formulations.

These and numerous other analogous, but often controversially discussed reports, on diverse bioactivities and bioactive constituents of *Withania somnifera* and other better scrutinize adaptogenic plants, led us to the working hypothesis that several often neglected secondary plant metabolites often encountered in *Withania somnifera* could as well be involved in their observed wide spectrums of therapeutically exciting pharmacological activity profiles. Although *Withania somnifera* is now often used in Ayurveda for prevention of stress triggered pathogenic conditions, it is not yet very certain whether and which biological processes and mechanisms other than those regulating stress responses are involved in their observed therapeutic benefits against mental disorders commonly associated with or caused by metabolic disorders. Such knowledge gaps open avenues to conduct more systematic studies necessary not only for better understanding of pharmacological principles behind the traditionally known medicinal uses of the plant but also for obtaining drug leads from the plants. Some of the fundamental questions that have to be more rationally answered for such purposes are:

1. Do all parts of the plant possess adaptogenic activity? If yes, what are the relative potencies and pharmacological activity profiles of different types of extracts obtained from different parts of the plant cultivated, harvested and processed under different conditions?

2. Whether or not the therapeutically interesting pharmacological activity profiles of the extracts of the plant devoid of withanolides, are qualitatively and quantitatively comparable to those of extracts enriched in withanolides?

3. What are the pharmacological targets and physiological processes involved in the modes of action of the therapeutically used extracts of *Withania somnifera* and their bioactive constituents*?*

Since numerous Ayurvedic and other traditionally known medicinal plants and their formulations possess adaptogenic, or stress response suppressing activities, in the realm of psychopharmacological studies based on Ayurvedic therapeutic principles [S.S. Chatterjee and V. Kumar, 2012], several rodent bioassays and experimental procedures for detecting, quantifying, and comparing adaptogenic potentials of herbal extracts and their bioactive constituents have been conceived and pharmacologically validated in our laboratories [G.M. Husain et al., 2011; A.K. Thakur et al., 2013a; 2013b; 2014a; 2014b; 2014c; A.J. Langstieh et al., 2014]. In principle, these bioassays are based on the well-known effects of adaptogenic herbal extracts on physiological processes regulating body temperature of rodents subjected to diverse stressful stimuli. Such effects of numerous adaptogenic herbs have been known since long, and diverse rodent models experimental procedures now often used to detect adaptogenic potentials of medicinal herbs and their formulations are based on their modulating effects on thermoregulatory physiological processes [U. Ramachandran et al., 1990; R. Kumar et al., 1996; V.S. Pawar and H. Shivakumar, 2011]. It has since long been well recognized that thermoregulation is impaired in metabolically abnormal and aged patients [A.R. Scott et al., 1987; J.E. Yardley et al., 2013; F. Khan et al., 1992], and that numerous so-called lifestyle diseases and physical and mental health problems of the elderly population often accompany metabolic disorders. Although numerous adaptogenic herbs are often used in the Ayurvedic system of medicine for prevention and treatment of diverse such conditions, the question whether or not their modulating effects on thermoregulatory

processes are also involved in their therapeutic benefits often observed in patients suffering from diverse co-morbid mental health problems still remain open, or speculative only.

Numerous more recent observations made in our laboratories and elsewhere strongly suggest that such could indeed be the case for numerous adaptogenic herbs, and that stress response suppressing effectiveness of herbal extracts (adaptogenic or not) increases not only with their doses but also with the numbers of days of treatments and the time elapsed after their last treatment and observations made [V. Kumar and S.S. Chatterjee, 2014]. That such is also the case for commercially available withanolides containing *Withania somnifera* extract is indicated by the observation made during efforts to verify the role of fumaric acid and its esters in the stress response suppressing effects of another Ayurvedic medicinal herb *Fumaria indica* [A. Shakya et al., 2016]. In that study, a randomly selected fairly high daily oral dose (100 mg/kg/day) of the extract was used as a reference standard for reaffirming the reproducibility of the experimental procedure used. Results of that study indicate also that the high daily oral doses of the extract for several days are well tolerated by rodents and that this is most probably the maximally effective oral doses of the tested extract for suppressing numerous stress responses triggered by occasional exposures to a very short duration of aversive stimuli.

Drug discoverers and developers have well recognized that translation of available preclinical and clinical knowledge on bioactive substances for medicinal or healthcare purposes, or for better understanding therapeutic mechanisms, it is necessary to have appropriate knowledge on their quantitative systems pharmacology [D.R. Abernethy et al., 2011]. Preclinical and clinical information now available in PubMed and databases on diverse types of *Withania somnifera* extracts and numerous of their known bioactive constituents strongly suggest that appropriate combinations of bioactive substances encountered in them could as well be used for prevention and cure of metabolic and/or environmental stress-triggered diseases, illnesses,

and central sensitivity syndromes accompanying or caused by repeated exposures to unpredictable stressful situations. However, as yet only very little systematic efforts have been made to better understand quantitative systems pharmacology of *Withania somnifera* extracts. Similarly, except for a few withanolides, as yet little quantitative information on numerous other bioactive secondary metabolites of the plant is available.

Therefore, the ultimate goal of the experimental work reported in the thesis was to identify and pharmacologically validate a rodent bioassays that could be used for obtaining and manufacturing pharmacologically well characterized mixtures of withanolides and extracts of *Withania somnifera* that could be further developed as multi-targeted drugs, or as phytopharmaceuticals and nutraceuticals, potentially useful for prevention and cure of mental health problems commonly accompanying, or caused by, almost all so-called lifestyle disorders. Hereupon, most experimental procedures used were those well standardized and often used in our laboratories and elsewhere for preclinical studies with adaptogenic herbal extracts and their bioactive constituents. A few others used were also well standardizes and pharmacologically validated before using them for the experiments described.

Aim of the reported biochemical studies was to identify some easily quantifiable biomarkers that could be used for identifying appropriate cellular and other *in vitro* bioassays potentially useful for medicinal chemists, botanists and other researchers in herbal industries interested in obtaining pharmacologically better standardized extracts from different parts and cultivars of *Withania somnifera* and other adaptogenic plants well known for their medicinal and nutritional values. Such efforts will certainly be very useful for more reliable prediction of therapeutic potentials of diverse types of extracts in clinical trials often based on the uses of so-called "surrogate biomarkers" of diseases, or for ascertaining therapeutic potentials of plant extracts, their bioactive constituents, and their combinations. Some other potential uses

of the observations made by diverse experimental strategies followed in this thesis are discussed and pointed out in the discussion section of the thesis.