CHAPTER – 6

IMPLICATION FOR TRANSLATION RESEARCH

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It is now well recognized that that the quantitative knowledge on systems pharmacology of drug leads is essential for discovering drugs and understanding therapeutic [M. Williams, 2011; P.K. Sorger et al., 2011] and that biological interactions between structurally and functionally divers bioactive constituents of herbal extracts dictate their pharmacological activity and safety profiles [T. Efferth and E. Koch, 2011; J. Gertsch, 2011; M. Wink, 2008]. Although available preclinical and clinical observations do add considerable evidences in support of the traditionally known medicinal uses of diverse types of pharmaceutical formulations of *Withania somnifera* derived products for prevention and cure of mental health problems, many questions concerning bioactive constituents of diverse types of extracts of the plant and their modes of action still remain open or speculative only. Analogous is the situation for all pharmacologically polyvalent phytochemicals and other naturally occurring substances and drugs derived from them.

Withania somnifera, like all other plants, biosynthesizes and store diverse combinations of structurally and functionally diverse phytochemicals for their own survival and defense against environmental stress [D.O. Kennedy, 2014b; D.O. Kennedy and E.L. Wightman, 2011; K.K. Vinod, 2012]. Although current practices to standardize its extracts on their withanolides contents can guarantee the authenticity of the starting materials used for obtaining them, several reports revealing and reaffirming stress resistance increasing and other therapeutic potentials of *Withania somnifera* extracts devoid of withanolides and several other phytochemicals encountered in them have appeared during more recent years [B. Singh et a., 2001; 2003; R. Wadhwa et al., 2013; N. Shrivastava et al., 2015]. Therefore, the ultimate goal of the described experiments was to verify whether *in vivo* bioassay procedures using foot shock stressed rodents as experimental animals could also be used for

better understanding of quantitative systems quantitative system pharmacology of diverse types of *Withania somnifera* derived products, their bioactive constituents and their mixtures. Hereupon due attention was paid to the facts summarized in **Table 5.2** and still often neglected by modern drug hunters and phyto-pharmacologists.

Table 6.1: Some facts well recognized by modern biologists, physiologists, and pharmacologists, but often neglected by drug designers and others interested in better understanding of medicinal phytochemistry and phytopharmacology of herbal remedies.

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1) Various combinations of so called "food phytochemicals" [D.S. Wishart et al., 2009] are biosynthesized and stored in all terrestrial plants and can also contribute to their medicinally interesting bioactivity profiles of their extracts.

2) Plants biosynthesize and store phytochemicals not only for their nutritional demands, but also for protecting themselves from predators and stress [N. Pedrol et al., 2006].

3) Many phytochemicals altering brain functions do so by regulating the physiological functions of digestive system and autonomic nervous system [D.O. Kennedy, 2014b].

4) Gut microbial ecology plays a crucial role in dictating gut feelings and metabolic status of all animals necessitating plant derived food for survival, and health maintenance [D.O. Kennedy, 2014a; A.K. Thakur et al., 2014b; A.W. Janssen and S. Kersten, 2015; 2017; E.A. Mayer, 2011; C. Milani et al., 2016].

5) Observable acute dose effects of drugs and other bioactive substances are not very predictive of their therapeutic potentials or health effects, or pharmacological activity profiles, and could as well be opposite of those observed after their repeated doses [C. Page, 2011; V. Kumar and SS. Chatterjee, 2014; M.Z. Teixeira, 2013].

6) Modulation of stress responses by phytochemicals and drugs and their combinations is involved in their regulatory effects on metabolic processes and mental functions dictating health status of all living organisms including humans [V. Calabrese et al., 2012; M.M. Sthijns et al., 2016].

7) Preexisting allostatic load of a given individual dictates the effects of drugs, or of any therapeutic measure, on a given day and age of his life depends on his prior physical and mental health status formed by his eating habits and ability to adapt and react against diverse environmental challenges and mental stress [B.S. McEwen, 1998; 2000].

Results of the experiments reported in this thesis reaffirm that presence of withanolides in *Withania somnifera* extracts is not essential for obtaining therapeutic benefits from them, and that the bioassay procedures used and diverse biomarkers of stress responses easily quantifiable in rodents are well suited not only for obtaining therapeutic leads from *Withania somnifera* and other plants, but also for better understanding of quantitative systems pharmacology of pharmacologically polyvalent drugs and drug leads. They revel and reaffirm also that like metformin, fairly low daily oral doses of diverse types of *Withania somnifera* extracts increases oxidative stress resistance, and strongly suggest that their modulating effects on the so-called microbiota-gut-brain axis regulating glucose and insulin homeostasis are involved in their modes of actions.

Appropriate and inappropriate production of oxidants, together with the ability or inability of organisms to respond to oxidative stress, is intricately connected to all age and lifestyle associated physical and mental health problems [T. Finkel and N.J. Holbrook, 2000; B.C. Dickinson and C. J. Chang, 2011]. It is now apparent also that maladaptive biological processes are involved in pathogenesis and progression of all chronic diseases, including diabetes and associated comorbidities [D.N. Brindley and Y. Rolland, 1989; A. Mitra, 2008], and that structurally and functionally diverse food phytochemical commonly consumed with every day meals and herbal remedies [M. E. Saltveit, 2017] posses anti-oxidative properties, or can modulate the functions of digestive processes regulating oxidative status of living animals [R.K. Naviaux, 2012; D.G. Lindsay, 2005; M. Birringer, 2011]. The observations that qualitatively as well as quantitatively the activity profiles of WSR and WFWS are

almost identical, strongly suggest that the combination of such phytochemicals encountered in the plant dictate the quantitative systems pharmacology of its extracts, and that their pharmacological standardization in the bioassays used in the dose finding experiments could at present be a more rational approach for obtaining more reliable and reproducible health benefits from them.

Pharmacologists have since long been well recognized that for increasing reproducibility and predictability of preclinical observations in experimental animals it is necessary to pay due attention to their stress responses to experimental procedures and prior health status [H. Anisman and Z. Merali, 1999; V. Claassen, 2013]. Urgent necessity of reducing the frequency and severity of such problems has now been well recognized by drug developers and grant giving authorities [F. Prinz et al., 2011; F.S. Collins and L.A. Tabak, 2014]. More recently it has also been pointed out also that better understanding the role of gut microbiota in regulating physical and mental health is essential for increasing the possibility of success in translating preclinical observations for combating metabolic disorders and resolving the reproducibility problems in preclinical research [D. J. Drucker, 2016]. For such purposes, many food phytochemicals encountered in terrestrial plants and their combinations can be used as experimental tools. Since effectiveness of several such phytochemicals (ascorbic acid, lactic acid, quercetin, salicylic acid etc) encountered in *Withania somnifera* in increasing stress resistance in occasionally foot shocked rodents can be easily be quantified after their repeated fairly low daily oral doses [A.J. Langstieh, et al., 2014; S.A. Khan et al., 2015; N. Shivavedi, et al., 2014a; 2014b; N. Shrivastava, et al., 2015], better understanding of the role of gut microbiota in the observed effects in such animals could certainly be useful also for reducing reproducibility, and increasing predictive validity, of preclinical observation in translation research.

More recent reports on computer assisted literature searches on available information on bioactive substances isolated from *Withania somnifera* and their biological targets [N. Widodo et al., 2011] have identified 163 compounds, amongst which only 22 have been reported to be present in the roots of the plant [U. Chandran and B. Patwardhan, 2017; R. Kalra and N. Kaushik, 2002]. In total, 57 therapy relevant pharmacological targets for only 8 of these 22 compounds were identified in one of these reports [U. Chandran and B. Patwardhan, 2017]. Surprisingly though, this and several other analogous reports on known bioactives of other plants neglect the fact that diverse combinations fairly ubiquitous small molecular substances like fumaric, salicylic, nicotinic acids, and numerous other plant poly-phenolics and vitamins are also encountered in their extracts and that they could also contribute to their observed pharmacological activity profiles. On the other hand, there is no dearth of preclinical and clinical reports revealing and reaffirming diverse therapeutic potentials of numerous such substances commonly consumed not only with every day meals and often considered by herbal researchers as bioactive constituents of phytopharmaceuticals now often commercialized as nutraceuticals or nutritional additives or food supplements.

Despite availability of numerous psychoactive and other drugs, and increasing acceptance and popularity of alternative and complementary systems of medicine and health care, prevention and cure of diabetes and other metabolic disorders associated mental health problems still continue to be a major challenge for all health care authorities around the globe. Together with China, India, and other economically developing countries are the worst sufferers of such diseases and illnesses. Due socioeconomic and cultural reasons, a vast majority of population in these countries herbal remedies and medical advices of traditionally known systems still continue to be the major, and often the only, therapeutic options. Observations made with metformin and *Withania somnifera* extracts, taken together

with our current knowledge on health benefits of structurally and functionally diverse food phytochemicals, strongly suggest that at present appropriate combinations of the antidiabetic drug and pharmacologically well standardized *Withania somnifera* extracts could be a more realistic approach for combating the spreading epidemic of diabesity, i.e. malnutrition and sedentary behaviour associated type-2 diabetes [T. Barber, 2016; J.M. Pappachan and A. K. Viswanath, 2017; P.Z. Zimmet, 2017].

The more holistic pharmacological strategy and rodent bioassays used in the present study seems to be well suited not only for pharmacologically standardizing *Withania somnifera* extracts but also for identifying such appropriate combinations of metformin and other traditionally known medicinal and food plants often cued for helping patients suffering for diabetes and other slowly and silently progressing metabolic disorders. Observations made in diabetic rats strongly suggest that quantification of choline ester hydrolyzing enzymes in body fluids of patients can be used for translating available preclinical information on *Withania somnifera* and other so called "adaptogenic herbs" [D. Winston and S. Maimes, 2007] for the benefit of patients suffering from, or at risk to, such medical conditions.