COMPARISON OF THERAPEUTICALLY INTERESTING BIOACTIVITY PROFILES OF *WITHANIA SOMNIFERA* EXTRACTS IN RODENT MODELS

Thesis submitted in partial fulfillment for the
Award of Degree

Doctor of Philosophy

By

Amitabha Dey

DEPARTMENT OF PHARMACEUTICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI– 221 005
INDIA

Roll No. 13161001

2018

CHAPTER – 7

CONCLUSION AND OUTLOOK

CONCLUSION AND OUTLOOK

Scholars and practitioners of Ayurvedic and other traditionally known systems of medicine and health care have known since antiquity that appropriate choice of food and eating habits is essential for health maintenance and prevention and cure of diseases [S. Rastogi, 2014]. Numerous pharmaceutical formulations containing combinations of diverse types of products derived from food and adaptogenic plants are mentioned in classical Ayurvedic texts, and numerous currently commercialized Ayurvedic and other herbal formulations contain *Withania somnifera* extracts as one of their major active components. For such uses, the extracts are now often obtained by arbitrarily chosen processing and extraction procedures and standardized on their withanolides contents and chromatographic fingerprints using combinations of diverse analytical and quality control procedures recommended by health care authorities. As pointed out in the discussion chapter of this thesis, such practices are certainly necessary for guarantying the authenticity of the plant sample used for obtaining the extracts but are of limited value for judging the medicinal values of the plant or of their extracts. This is because the contents of their already known or unknown bioactive constituents vary considerably in different parts of the plant obtained from different cultivars and harvested in different seasons and processed under different conditions.

Observations reported in this thesis reveal and reaffirm though, that diverse types of *Withania somnifera* extracts, containing withanolides or not, possess stress resistance increasing activities even after their fairly low daily oral doses, and that all of them are almost equiactive in affording protection against stress triggered alterations in body weight and thermoregulatory processes. Therefore, one major conclusion of these observations is that pharmacological standardization of *Withania somnifera* extracts in bioassay procedures used for estimating their pharmacologically active doses and treatment regimen is more reliable for ascertaining their pharmaceutical quality than analytical standardization of the extracts on

their total contents in withanolides only. Some other more definitive conclusions of the observations made in the reported experiments can be summarized as follows:

1. Combinations of structurally and functionally diverse phytochemicals, and micronutrients encountered in *Withania somnifera* extracts are involved in their traditionally known stress resistance increasing and other medicinal uses, and that many food phytochemicals encountered in them play important roles in dictating their pharmacological activity profiles.
2. Parts of the plant other than roots can also be used for obtaining therapeutic benefits against diseases and illnesses triggered by chronic exposures to unavoidable and noxious environmental stimuli, and that such effectiveness of *Withania somnifera* extracts does not depend on the sex of the experimental animals used.
3. Repeated daily oral dose studies are necessary for better understanding quantitative systems pharmacology of *Withania somnifera* extracts and other drugs with stress response modulating activities and often prescribed for treatments of stress triggered health problems.
4. Therapeutically interesting bioactivity profiles and effectiveness of *Withania somnifera* extracts depend not only on its daily dosed used but also on the number of days of treatments administered.
5. Qualitatively, pharmacologically activity profiles of metformin and *Withania somnifera* extracts are quite analogous, but not identical. Metformin seems to be more effective in regulating glucose and insulin homeostasis than *Withania somnifera* extracts, whereas the extracts of the plant are more effective in affording protection against chronic stress triggered exaggerated states of anxiety and depression than metformin.

Although the reported observations do not allow very definitive conclusions concerning bioactive constituents, pharmacological target, and modes of actions of *Withania somnifera* extracts, they do suggest that modulation of the physiological functions of the microbiota-gut-brain axis is involved in their modes actions. Moreover, they suggest also that *Withania somnifera* extracts could be used as herbal alternatives for prevention and cure of eating disorders often caused by environmental stress and leading to diverse spectrums of physical and mental health problems, or for suppressing abnormal desire to consume palatable food by persons prone to obesity, diabetes, and other metabolic disorders. Further, more detailed studies will be necessary for reaffirming such possibilities.

In any case, it remains certain that presence of withanolides in *Withania somnifera* extracts is not necessary for obtaining therapeutic benefits from them, and that the bioassay procedures used in this study, and the biomarkers quantified in them, are well suited not only for better understanding of quantitative systems pharmacology of the plant but also for obtaining novel therapeutic leads from them. However, continuous validation of these bioassay procedures will be necessary to develop a convenient bioassays system universally acceptable for detecting and quantifying stress response suppressing potentials of diverse types of extracts obtainable from *Withania somnifera* like adaptogenic plants and their bioactive constituents.

Knowledge and knowhow evolving from efforts to better define medicinal phytochemistry and quantitative systems pharmacology of *Withania somnifera* derived product will certainly be useful for obtaining therapeutic leads from other plants often used in Ayurvedic and other traditionally known systems of medicine as tonics and rejuvenators, or as adaptogens. This is because many phytochemicals, including withanolides, are also biosynthesized and stored in different combinations in all plants. Observations in our laboratories and elsewhere have often reaffirmed that numerous of them posses stress response modulating and other therapeutically interesting bioactivities even after their fairly low daily oral doses.

Although available information on oral bioavailability (as judged by their blood levels observed after oral administration) of known phytochemicals (many of which are now often collectively referred to as food phytochemicals) encountered in *Withania somnifera* are almost negligible, the observed activity profiles of all *Withania somnifera* extracts in the bioassay systems used were almost identical to that of metformin, and quite analogous to those of salicylic acid, fumaric acid and quercetin studied in more details in our laboratories. Since stress triggered elevation of choline esterase activities in blood and brain regions of diabetic rats were completely absent in *Withania somnifera* extracts treated diabetic rats, it can safely be concluded also that biological processes and mechanisms involved in regulating systemic functions of choline esters are involved in modes of actions of *Withania somnifera* extracts, metformin and many other food phytochemicals commonly consumed with everyday meals or with phytopharmaceuticals, currently often commercialized as nutraceuticals. There is no dearth of reports revealing and reaffirming the involvement of choline esterases in diabetes associated physical and mental health problems [G.I. Lunkes et al., 2006; M. Cucuianu, et al., 2001; L. Santarpia et al., 2013].

Toxicologists and experimental pharmacologists have since long well recognized that for increasing the reproducibility and predictive validity of preclinical observations due attention has to be paid to the variability of biological responses in different groups of experimental animals subjected to diverse stressful experimental procedures necessary for conducting the experiments [V. Claassen, 2013; J. Hau and S. J. Schapiro, 2002], and that alteration in choline esterases activity are a reliable indicator of diverse stress responses [B.A. Rattner and A. Fairbrother, 1989; K.C. Parsons et al., 2000]. More recently it has often been pointed out also that irreproducibility of observations in laboratory animals can arise from variation in diets of animals, shuttle handling issues, and diverse environmental factors [S. Reardon, 2016; R. Fodde, et al., 2017]. Observations reported in this thesis add further experimental

evidences justifying the uses diverse types of combinations of fairly low doses of *Withania somnifera* extracts with other health promoting ingredients for prevention and cure of diverse so-called lifestyle disorders associated mental health problems, and suggest that appropriate uses occasionally foot shock stressed rodents could be used for increasing the reproducibility and predictive validity of pharmacological and toxicological models necessary for drug discovery and development purposes. Abnormal changes in growth rates, physiological thermoregulatory process and obsessive compulsive behavior, and exaggerated states of anxiety, depression are common symptoms of numerous so-called "lifestyle disorders", and observations in our laboratories have reaffirmed all these disease symptoms can be conveniently and reproducibly quantified in rodents repeatedly exposed to unpredictable noxious stimuli for even less than one minute in different groups of animals and in all seasons by observers of different ages and sexes.

Since global commercial demand and popularity of medicinal and healthcare uses of the plant have continued to increase during more recent decades, efforts are now being made in many laboratories to identify diverse agricultural and other biotechnologies necessary for meeting the commercial demand and increasing the yields of *Withania somnifera* derived medicinal products [P. Singh et al., 2017]. Possibility of successes and failures of such ventures for medicinal and health care purposes will depend largely on the pharmaceutical quality and medicinal vales of the final products. Availability of well standardized and reproducible *in vivo* bioassays with reasonable predictive validity is an essential prerequisite for guaranteeing and more reliably predicting such qualities and therapeutic potentials of all plant derived product. This is because, all such products (including pure substances isolated from them) are pharmacologically polyvalent and their metabolic fate, bio-availability, and bio-accessibility will depend largely on the nutritional status and gastro-intestinal contents of all substances consumed by the patient with every day meals and drinks. These facts, taken together with our

current knowledge on nutritional and health care values of food phytochemicals, strongly suggest that the pharmacological strategy and bioassays used in the experiments described in this thesis are well suited not only for pharmacological standardizations of different types of plant extracts, but also for more rational and evidence based medicinal uses of plant derived products and almost all pharmacologically polyvalent drugs and phytochemicals and drug leads.

Observations reported in this thesis suggest that the bioassay procedures used in the experiment are particularly well suited for identifying poly-pills against diabetes associated mental health problems and other comorbidities, and that appropriate combinations of *Withania somnifera* extracts with other antidiabetic/psychoactive drugs could as well be a promising and more reasonable therapeutic option for diabetes associate co-morbidities. However, more detailed studies on rodents as well as human subjects will be necessary for reaffirming such possibilities. Efforts to identify the bioactive constituents of *Withania somnifera* extracts devoid of withanolides and their modes of actions using the bioassays used could eventually lead to identification of novel pharmacological targets and therapeutic leads urgently needed for prevention and cure for environmental stress and lifestyle associated mental health problems. In any case, such efforts will certainly be useful for more rational translation of Ayurvedic epistemology [G. Basisht, 2014; A. Delle Fave et al., 2015] in terms of modern life sciences, and evidence based integration of traditionally known herbal remedies in modern medical practices [U. Chandran et al., 2015; H. Li et al., 2014; J. Li et al., 2012; W. Li et al., 2017].

Therefore it is apparent that the observation made by uses of the pharmacological and medicinal phytochemistry strategy, bioassays, and reference drugs and plant extracts used in this thesis is not only well suited for better understanding of Ayurvedic pharmacology of medicinal and food plants, but also for increasing the possibility of success in all drug

discovery and development ventures necessary for combating the health problems of the 21st century.