Reverse Pharmacology and Systems Approaches for Drug Discovery and Development

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Abstract: While biotechnological advances, genomics and high throughput screenings or combinatorial and asymmetric syntheses have opened new vistas in drug discovery, the industry is facing a serious innovation deficit. Critics suggest that "we have become high throughput in technology, yet have remained low throughput in thinking". Post marketing failures of blockbuster drugs have become major concerns of industries, leading to a significant shift in favor of single to multi targeted drugs and affording greater respect to traditional knowledge. Typical reductionist approach of modern science is being revisited over the background of systems biology and holistic approaches of traditional practices. Scientifically validated and technologically standardized botanical products may be explored on a fast track using innovative approaches like reverse pharmacology and systems biology, which are based on traditional medicine knowledge. Traditional medicine constitutes an evolutionary process as communities and individuals continue to discover practices transforming techniques. Many modern drugs have origin in ethnopharmacology and traditional medicine. Traditions are dynamic and not static entities of unchanging knowledge. Discovering reliable 'living tradition' remains a major challenge in traditional medicine. In many parts 'little traditions' of indigenous systems of medicine are disappearing, yet their role in bioprospecting medicines or poisons remains of pivotal importance. Indian Ayurvedic and traditional Chinese systems are living 'great traditions'. Ayurvedic knowledge and experiential database can provide new functional leads to reduce time, money and toxicity - the three main hurdles in the drug development. We begin the search based on Ayurvedic medicine research, clinical experiences, observations or available data on actual use in patients as a starting point. We use principles of systems biology where holistic yet rational analysis is done to address multiple therapeutic requirements. Since safety of the materials is already established from traditional use track record, we undertake pharmaceutical development, safety validation and pharmacodynamic studies in parallel to controlled clinical studies. Thus, drug discovery based on Ayurveda follows a 'Reverse Pharmacology' path from Clinics to Laboratories. Herein we describe such approaches with selected examples based on previous studies.

Keywords: Reverse pharmacology, Drug discovery, Adjuvants, Chemoprotection, Adaptogens, Ayurveda.

INTRODUCTION

The pharmaceutical sector has traditionally been a vibrant, innovation-driven and highly successful component of global industry. A confluence of spectacular advances in chemistry, molecular biology, genomics and chemical technology and the cognate fields of spectroscopy, chromatography and crystallography led to the discovery and development of numerous novel therapeutic agents for the treatment of a wide spectrum of diseases. To facilitate this process, scientists launched a significant and noticeable effort aimed at improving the integration of discovery technologies, chemical sourcing for route selection / delivery of active pharmaceutical ingredients. Drug discovery and development was increasingly done sans frontiers with collaborations spanning the globe and utilizing scientists with a broad array of technical, professional and cultural boundaries.

The pharmaceutical industry has historically seen incredible growth due primarily to the industry's strategy of focusing efforts towards development of "blockbuster" drugs with the potential to generate over $1 billion in sales. However, recent trends indicate that this model may no longer ensure high growth rates [1]. The average cost of discovering, developing and launching a new drug in June 2008 was inordinately high and represented a dramatic increase over the average cost from 1995 [2]. R&D expenses have risen from $2 billion in 1980 to over $40 billion in 2007. Surprisingly, these increases have not led to a corresponding increase in the number and efficacy of new drugs. From 1995-2000 (as compared to the previous five years), the number of New Molecular Entities (NMEs) approved dropped by nearly 50 percent, to about 40, and the number of New Chemical Entities (NCEs) produced per company declined by 41 percent. Moreover, the number of approvals for New Molecular Entities (NMEs) has steadily declined reaching a low of 17 in 2002 and even lower to less than 10 in 2007 and 2008 [3]. High throughput assays capable of interrogating individual molecular targets with a number of compounds are used to speed up screening processes enormously. Yet, numbers of approved new chemical/ molecular entities are declining-regulatory agencies have increasingly changed clinical end-points and have advocated against "me-too" drugs. The situation is progressively deteriorating [4].

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The industry in the U.S.A. and Europe is currently undergoing unprecedented changes. The introduction of fewer NCE's allied with the expiration of patents on blockbuster drugs (thereby conferring generic status on them and resulting in a precipitous drop in sales revenues) has resulted in increased collaborations at the academia / industry interface. Approximately $30 billion of pharma-ceuticals will come off patent. The rapidly increasing pace of regulatory reform allied with the necessity of effecting drastic reductions in costs have also resulted in marked shifts in the strategic paradigms in this industry. Challen-ges that face the industry primarily are those of sustaining growth and profitability with ever increasing research costs. Numerous corporations are seeking strategic partnerships overseas. Pre-requisites like a highly trained and motivated work force, political stability, and the capability driven ability to innovate are sine qua non. Many analysts believe that the current model— "the one drug to fit all", approach will be unsustainable in future and that a new model is necessary for further scientific growth. A new chemical entity (NCE) travels a path from laboratories to clinics, involving target identification, lead identification, lead optimization, preclinical studies, and then four phases of the clinical trials: a 12-14 years odyssey is usual!. The extremely complex and capital-intensive process makes companies 'target rich' but 'lead poor'. Multi-target approaches are coming into the main stream [5]. Alternative and Comple-mentary approaches in therapeutics are becoming popular options.

Modern or Conventional medicine is as practiced by medical doctors and by their allied health professionals, such as physical therapists, psychologists, and registered nurses. Other terms for conventional medicine include allopathy; Western, mainstream, orthodox, regular medicine; and biomedicine. Complementary and alternative medicine (CAM) is a group of diverse medical and health care sys-tems, practices, and products that are not presently consi-dered to be part of conventional medicine. Traditional medicines are largely based on medicinal plants, indigenous to these countries: effort is focused on accessing them either directly or through the use of modern tools of breeding and cultivation, including tissue culture, cell culture and transgenic technology.

Historically, the terms alternative, complementary or traditional medicine (TM) all referred to a genre of health care practices or services that were grouped as a class through the logic of reductio-ad-absurdum, or because of their "absence from the mainframe of" Modern Medicine (MM). WHO defines traditional medicine as including diverse health practices, approaches, knowledge and beliefs incorporating plant, animal, and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness.

The phraseology MM/TM is syntactically incorrect because it is contended that medicine, modern or otherwise, evolves and progresses through traditions. The major difference between the various traditions of health care practices is that MM rode the crest of the high wave of reductionist-scientific rigor of modernity [6] to reach the shore of global acceptability while the others never crossed the threshold of geographical and cultural locality, remaining untested, neither proved nor disproved. Darwinian principle of survival of the fittest also endorsed a truism that survival was a proof of fitness. TM as we know of today has holistic view for promoting human health as it considers human beings and health in the context of their environment. Developmentally, MM isolated "human" from its global environmental context as a static, stand-alone, complete, total and delimited system in itself. While this reductionist approach has a very high pay off for the majority of human health problems, it fails when dynamic non-linear complex systems elements are considered. TM covers some parts of those hidden varia-bles and confers benefit and utility to modern mankind. Integrative approaches, which take best of the available without bringing hierarchies or even intentions to undermine any, are becoming more acceptable. Holistic or Integrative Medicine (IM) is a comprehensive term that integrates traditional and modern systems of medicine.

Integrative medicine combines mainstream medical therapies and CAM therapies for which there is some high-quality scientific evidence of safety and effectiveness. The National Institutes of Health, USA has recognized the urgent need to bring all the CAMs into the mainstream of medicine. The National Center for Complementary and Alternative Medicine (NCCAM) has been inaugurated as the United States Federal Government's lead agency for scientific research in this arena. Its mission is to explore comple-mentary and alternative healing practices in the context of rigorous science, support sophisticated research, train researchers, disseminate information to the public on the modalities that work and explain the scientific rationale underlying the discoveries. The center is committed to exploring and funding all such therapies for which there is sufficient preliminary data, compelling public health need, and ethical justifications to do so.

Many cancer patients routinely use CAM as a primary treatment to alleviate the discomfort of conventional cancer therapy. 85 percent of cancer patients recently surveyed used at least one CAM therapy while undergoing conventional oncology treatment. Some oncologists treat cancer patients with dietary supplements, including pancreatic enzymes, magnesium citrate, papaya extracts, vitamins and minerals. Very preliminary data suggests the therapy may be effective in prolonging life expectancy of those suffering from pancreatic cancer. NCCAM has embarked upon a creative, new research grant mechanism to support well-designed studies in such areas as complex high dose anti-oxidants, herbal mixtures, and whole plant extracts. The Quick Trial mechanism has been used for pilot, phase I, and phase II cancer clinical trials testing new agents, as well as patient monitoring and laboratory studies to ensure timely development of new treatments.

AYURVEDA - THE ANCIENT SCIENCE OF LIFE

Ayurveda, the ancient life science, has a history of over 4000 years of practice. It is a great living tradition that addresses health with a unique holistic approach. Currently, with over 400,000 registered Ayurveda practitioners, the Government of India has formal structures to regulate issues

related to quality, safety, efficacy and practice of herbal medicine [7]. Ayurvedic medicines are usually customized to an individual based on their constitution. Ayurvedic philosophy strongly believes that human being is a microcosm of nature and all the five basic elements of nature are present in him. The original philosophical basis of Ayurveda is in Sankhya, which means "knowing the truth". The five basic principles or *Panchamahabhootas*, loosely translated as Earth, Water, Fire, Air and Ether are believed to constitute animal, plant and minerals as also the human body. Their relative proportions determine the constitution or *Prakriti* of an individual and is presented in form of three humors or *Doshas* namely *Vata* (Air and Ether), *Pitta* (Fire and Water) and *Kapha* (Water and Earth). *Prakriti* determines the physical as well as mental characteristics of an individual. These characteristics are given importance while deciding a therapeutic regime. Permutations and combinations of influencing factors including about 111 of *Vata*, 86 of *Pitta* and 92 of *Kapha* give near 800,000 variables. Thirty six other variable factors like desha (habitat), kala (time) and such takes this number to over 3,000,000. With additional confounding factors based onconcept of *dhatu sarata* and 20 *Guna* this number tends to infinity. Therefore according to Ayurveda each individual is different and must be treated only after consifdering the nature of their constitution or the Ayurvedic concept of *Prakriti*. Since this is extremely complex process, a decision support system based on Ayurveda knowledge base known as 'AyuSoft' has been developed by the Center for Advanced Computing (CDAC). AyuSoft [8] is a comprehensive, authentic, interactive, intelligent and communicative software to assist medical practitioners, researchers and health seekers and providers to apply basic principles of Ayurveda to the fullest possible level in their routine clinical practice. The concept of *Prakriti* has a central role in understanding health and disease in Ayurveda, which is very similar to pharmacogenetics that is expected to become basis of designer medicine. A preliminary proof for understanding of genetic basis and its correlation with Ayurvedic concept of *Prakriti* has been reported by suing HLA gene polymorphism [9]. A national project on AyuGenomics to study genomic variation and gene expression profiling of human *Dosha Prakriti* based on principles of Ayurveda'is underway where gene expression profiling, SNP based genotyping, data validation by DNA sequencing, STR-based genotyping and polymorphism in few selected genes such as P450, MDR, GST, NAT & MCR are being attempted [10].

Thus basic principle of Ayurveda is based on personalized approach can be used for creating personalized, customized or designer medicines. Therefore science of Ayurveda has the potential to revolutionize modern medicine and drug discovery processes. The systems biology approach based on Ayurveda knowledge will furnish two advantages: first, a systematization of very complex process of Ayurvedic therapeutics for uniform global clinical applications and second, an organized database of extract-activity libraries that will increase chances of better and functional leads. Integrated model based on AyuSoft and AyuGenomics will facilitate harmonizing and customizing medical practice and will be a powerful discovery engine for new drugs discovery. We believe and strongly recommend that this knowledge base of Ayurveda and traditional medicine must be complemented by certain principles of the systems biology and reverse pharmacology approach.

SYSTEMS BIOLOGY

Traditional medicines have been developed through real life experiences and direct observations in people with diseases and represent highly complex biological systems. Typical drug development incorporates preclinical studies on animal models, cells and tissue screens. This situation calls for a critical assessment of current strategies. Molecular biology based technologies are primarily used for defining molecular targets and for creating new miniaturized screening tests. Such reductionism where a whole organism is broken down into groups of tissues, cells, molecules and molecular interactions undermines the importance of the whole system. While interacting with each other, macromolecules form complex networks and get organized into systems with properties that extend beyond individual functions. Systems biology attempts to provide predictive models of behavior of diverse molecular systems to identify such functional interactions.

Current strategy of drug development of single target - single compound, is based on a super reductionism that involves molecular level assays [11]. This approach is not suitable for studies on traditional medicines. A more holistic approach using systems biology seems much more suited to probe and confirm the efficacy and understand the mode of action.

Systems approach or science of wholeness is the unique philosophy of Ayurveda. When multiple cell types and diverse pathways contribute to the disease, a single molecule may not be effective in modulation of multiple targets and such conditions require combination therapy. Herbal extracts represent combinatorial chemistry of nature with vast array of chemical compounds that can deal with multiple targets simultaneously leading to synergistic systems effect. Molecular technology such as microarrays can be deployed for testing herbal drugs that work in systems to arrive at their targets [12, 13].

We are now witnessing the entry of a new informational paradigm into medicine that is most prominently represented by proteomics, metabolomics sciences. Metabolomics is a systems approach for studying metabolic profiles, which promises to provide information on drug toxicity, disease processes and gene function at several stages in the discovery process. Spectroscopic and chromatographic methods are used for identifying and characterizing certain metabolic changes in human, then integrating the data outcome by bioinformatics and creating a metabolic profile for a single individual. Such integration of data types paves the way to understanding the relationships between gene function and metabolic control in health and disease. Systems biology thus offers the computational integration of data generated by the suite of genetic, transcriptomic, proteomic and metabolomic platforms to understand function through different levels of biomolecular organization [14]. This approach offers exciting new prospects for determining the causes of human disease and finding possible cures [15].

This paradigm could bring two important changes in the therapy of diseases. First, mature science will study complex genomes and their functionality in complex organisms such as humans. Secondly, drug therapy that used to be largely symptomatic, will now aim at targets emerging from systems closer to the causes of diseases. Therapeutic progress, which used to be indirect, conjectural and coincidental, will become more directed, definitive and intentional. Future drug discovery will be more often based on intent rather than coincidence. Proper bioprospecting to get real selectively medicinal sources and exclusion of potentially poisonous sources still remains an important factor [16].

REVERSE PHARMACOLOGY (RP)

Sir Peter B. Medawar rightly stated that "A synthetic discovery is always a first recognition of an event, a phenomenon, process, or a state of affairs not previously recognized or known. Most of the stirring and deeply influential discoveries of science come under this heading". Clinical events or phenomena previously not reported and following the administration of a known or new drug, can provide valuable insights for drug development. Medicinal plants and natural products derived there from have provided many such serendipitous bedside observations. Historically, several such clinical hits were not often pursued quickly and rigorously by the drug discovery teams. Similarly, research in genomics, proteomics and metabolomics has stimulated discovery of many new entities, which are yet to be pursued for their drug-like activities. A new trans-disciplinary endeavor called Reverse Pharmacology has recently emerged and addresses both these needs. Reverse Pharmacology (RP), designed as an academic discipline to reduce three major bottlenecks of costs, time and toxicity. RP can be perceived to comprise of three phases. First, the experiential phase that include robust documentation of clinical observations of the biodynamic effects of standardized Ayurvedic drugs by meticulous record keeping. Second, the exploratory studies for tolerability, drug-interactions, dose-range finding in ambulant patients of defined subsets of the disease and para-clinical studies in relevant *in vitro* and *in vivo* models to evaluate the target-activity. Third phase includes experimental studies, basic and clinical, at several levels of biological organization, to identify and validate the reverse pharmacological correlates of Ayurvedic drug safety and efficacy. The scope of reverse pharmacology is to understand the mechanisms of action at multiple levels of biology and to optimize safety, efficacy and acceptability of the leads in natural products based on relevant science. In this approach as the candidate travels a reverse path from 'clinics to laboratory' rather than classical 'laboratory to clinics' (Fig. **1**).

Actual human use as the ultimate model and in-depth investigation of the effects of drugs and the nature of disease progression is becoming ever more feasible because of advances in clinical biomarkers and systems biology. This articulates both structure of the system and components to play indispensable role forming symbiotic state of the whole system. Drug development for herbal drugs can follow different paths explained in the Fig. **2**.

The ethno medicine path based on observing field use of plants and then standardizing for the activity e.g. tubocurarine isolated from Curare - commonly known as South American arrow poison. Another route is through screening of extracts in selected models, isolation of the active principles and then definition of a single molecule, for preclinical and clinical evaluation such as reserpine from *Rauwolfia serpentina.* The herbal product path of screening of extracts in models and then testing for efficacy and safety in a clinical set up such as *Ganoderma lucidum* and *Hypericum perforatum*. The holistic path of using the formulation mentioned in classical texts of Ayurveda and then evaluating

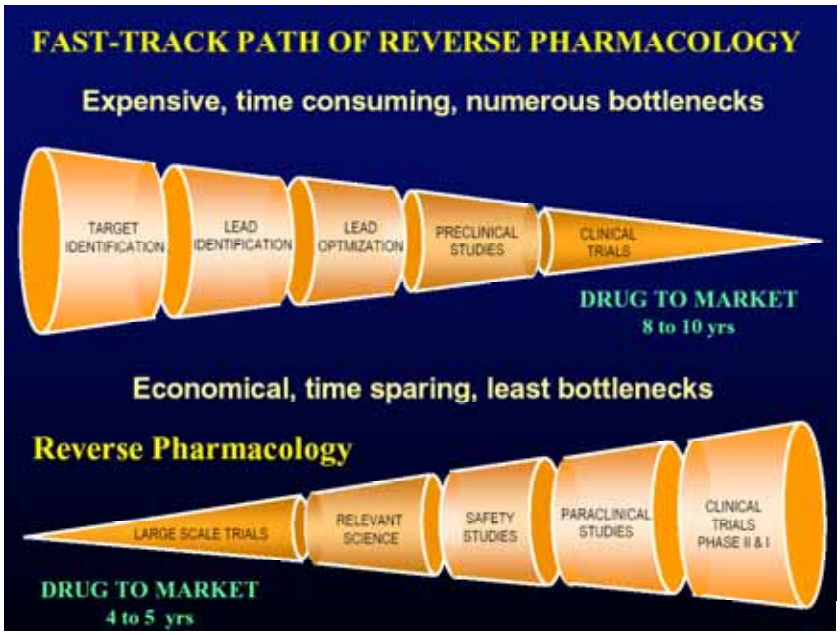


Fig. (1). Reverse Pharmacology Path.

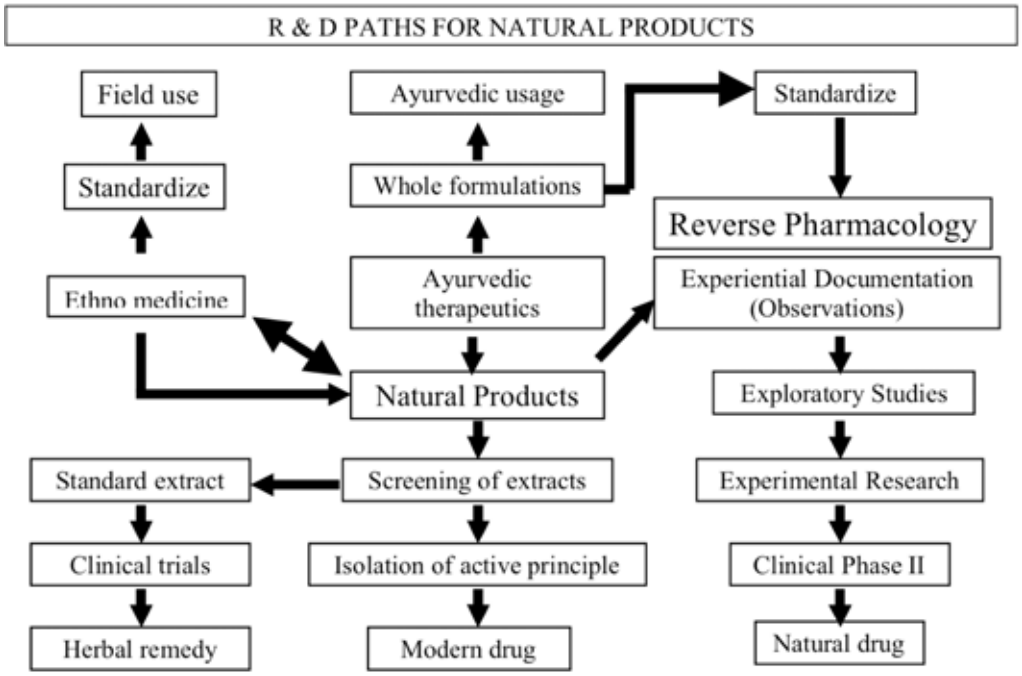


Fig. (2). R & D Paths for natural products.

efficacy of popular medicines in use such as *chavanprash* through pharmacoepidemological studies.

These paths have been thoroughly explored for years and have yielded effective medicines. Although botanical medications continued to be produced in every country, their clinical efficacy was usually not evaluated and the composition of these complex mixtures was only crudely analyzed. Investments in these methodologies remained scarce. Main factors contributing to such slow-tracking include failure to distinguish folklore from traditionally established systems like Ayurveda and TCM, lack of proper identity and implementation of Good Laboratory Practices, improper experiential documentation, absence of Phase II dose optimizing studies, cultural prejudice for alien sciences, emphasis on the reductionist path, and lack of political and financial support. Plants are compositional complex mixtures of chemicals and studying them is a challenge. An open-ended approach, to guide us from the traditional wisdom of Ayurveda to the current state-of-art developments in biology is being formulated. Such an integrative and interdisciplinary approach is the key to understanding the breadth of the drug action resurrect natural products based drug discovery

The credit for stimulating interest of Indian chemists and pharmacologists in medicinal plants should rightfully go to Sir Ram Nath Chopra who has been acclaimed as the 'Father of Indian Pharmacology" [17]. Gananath Sen laid the foundation of Reverse Pharmacology of medicinal plants by pursuing clinically documented effects of Ayurvedic drugs [18]. *Rauwolfia serpentina* Benth, was a major discovery through this approach. Sen and Bose not only convincingly demonstrated the antihypertensive and tranquillizing effects of the plant but also observed unique side effects such as depression, extra pyramidal syndrome, gynecomastia and such [19]. It took decades to delineate mechanisms of these side effects. This was a watershed for new antidepressants, anti Parkinson's drugs and prolactin-reducing drugs [20]. In the west, pharmacology as a discipline grew rapidly when the plants toxic to humans such as strychnos alkaloids, physostigmine, curare were studied for the mechanisms of action [21, 22].

Reverse pharmacology offers a major paradigm shift in drug discovery. Instead of serendipitous findings pursued randomly an organized path from clinical observations and successess is established. The science has to integrate documented clinical and experiential hits into leads by interdisciplinary exploratory studies on defined targets *in vitro* and *in vivo* and conducting the gamut of developmental activities. Recently, India has amended the Drug Act to include a category of phytopharmaceuticals to be developed from medicinal plants by Reverse Pharmacology, with evidence of quality, safety and efficacy. These drugs will be distinct from traditional medicines like Ayurvedic, Unani or Siddha. India with its pluralistic health care system offers immense opportunities for natural product drug discovery and development based on traditional knowledge and clinical observations [23]. A New Millennium for Technological Leadership Initiative (NMITLI) has resulted in hits, leads and effective formulations for diabetes, arthritis and hepatitis with novel mechanism of action and intellectual property [24] as shown in Table **1**.

Malaria, despite availability of a large number of chemotherapeutic agents, takes a severe toll in terms of mortality and morbidity. Common antimalarial drugs derived from plants include quinine and artmisinine. Recently, *Nyctanthes arbor-tristis* Linn has been shown to possess antimalarial activity. In a study in 120 patients, ninety-two (76.7%) showed complete parasite eradication and clinical cure within 7 days of treatment with the leaf paste of *N.*

Table 1. Reverse Pharmacology Correlates

Diabetes mellitus	Osteoarthritis	Hepatitis
• GLUT-4 translocation	• Chondrocyte protection	• Hepatoprotective- Anti TB drugs
• AKT-phosphorylation	• Disease-modifying	• Proinflammatory cytokines decrease
• Inhibition of glycation	• Muscular Strength	• Protection- Paracetamol
• Cataract prevention	• MRI improvement	• Reduction in fatty infiltration

arbor-tristis [25]. The plant extracts are being standardized and studied phytochemically as exploratory studies have already shown antiplasmodial effects *in vitro* and disease-modifying activity in patients [26]. This work has now been taken up by the ICMR Advanced Centre of Reverse Pharmacology in Traditional Medicine, in collaboration with the Centre of Molecular Parasitology at the Drexel University College of Medicine.

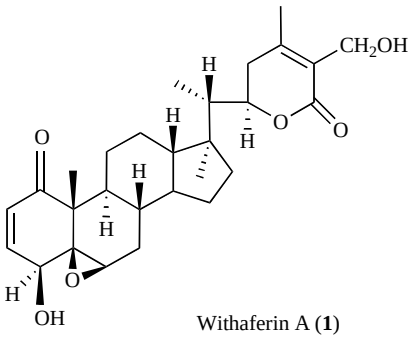
There is a need to develop an academic niche for Reverse Pharmacology in medical and pharmaceutical sciences colleges and drug R & D centers. Linkages must be established with Observational Therapeutics and Ayurvedic Pharmacoepidemiology to identify clinical hits. In India, major endeavors in this direction have been initiated already, both in the private and the public sectors of pharmaceutical R & D [27]. We give few examples from research and treatments for difficult to treat conditions including cancer, arthritis as case studies where systems biology and reverse pharmacology principles have been used to fast track drug discovery and development projects. Key active compounds from traditional medicine sources could serve as good starting compounds and scaffolds for rational drug design. Most of these compounds are part of routinely used traditional medicines and hence their tolerance and safety is assumed to be better than any other chemical entities that are totally new for human use

CASE STUDIES: DRUG DISCOVERY

Rauwolfia Serpentina (Sarpagandha)

Ganath Sen and Bose not only showed the antihypertensive effects of Sarpagandha but they were also astute clinicians to note certain side effects like parkinsonism, depression, gynecomastia, acid-peptic symptoms etc. It was only recently that, a Nobel Prize in Medicine and Physiology was given to those who validated and explained the actions by mechanistic correlates. As a spin-off of the side effects of *Rauwolfia serpentina*, several new drugs were developed viz. L-dopa, antidepressants, bromoergocriptine, and H2-receptor blockers etc. The alkaloids of *Rauwolfia serpentina*, reserpine and ajmalcine, have served as research tools in many experiments. There are still some unanswered questions: does reserpine have more incidences of depression and/or extrapyramidal side effects than the standardized extract of the plant? It is worthwhile to apply combinatorial chemical methods for reserpine-derivatives, which do not cross the blood-brain barriers, so that depression is avoided as a side effect. The uptake of norepinephrine by isolated chromaffin granules, by inhibition of ATP-Mg^2 - dependent mechanism has to be studied freshly at the transcriptional level. The *kaphava-tashamak* (anti-inflammatory) properties of the plant described in Ayurveda too need an investigation. Reserpine extracts of the plant are used as Ayurvedic drugs. There is a need to conduct pharmacovigilance on Sarpagandha traditional formulations that are in use even today.

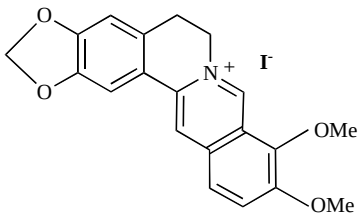
Withania somnifera (Ashwagandha): Drug derived from this is mentioned in the Indian Herbal Pharmacopoeia and Ayurvedic Pharmacopoeia [28]. Studies indicate that *Withania somnifera* (WS) modulated cyclophosphamide-induced toxicity. This reduction was in the terms of reversal of leuopenia, increase in bone marrow cellularity and reduction in CP associated urotoxicity. Towards pharmacological mechanisms, WS was found to enhance Interferon gamma (IFN-gamma), Interleukin-2 (IL-2) and Granulocyte macrophage colony stimulating factor (GM-CSF) which were lowered by Cyclophosphamide administration [29]. These studies indicate *Withania somnifera* could reduce cyclophosphamide toxicity [30]. WS was also shown to prevent lipid peroxidation (LPO) in stress-induced animals indicating its adjuvant well as chemoprotectant activity [31]. In another mechanistic study, WS was found modulatory on O6-methylguanine-DNA methyltransferase (MGMT), which is important for chemoprevention from alkylating agents induced O6 alkyl guananidines [32]. Glycowithanolides, consisting of equimolar concentrations of sitoindosides VII-X and withaferin A **(1)**, isolated from the roots of WS were evaluated for protection in iron induced hepatoxicity in rats. 10 days of oral administration of these active principles, in graded doses (10, 20 and 50 mg/kg), resulted in attenuation of hepatic lipid peroxidation (LPO), the serum enzymes alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase during iron-induced hepatoxicity [33]. Anti-stress activity observed with *Withania somnifera* promises to be an additional benefit along with chemo-protectant activity [34-37].



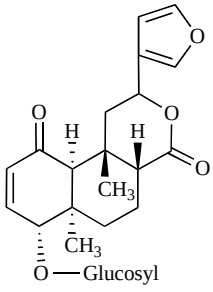
Withaferin A (**1**)

Tinospora cordifolia (Guduchi): Is widely used and is known for its immunomodulatory, antihepatotoxic, antistress and antioxidant properties. It has been used in combination with other plant products for a number of Ayurvedic preparations. The chemistry has been extensively studied and its chemical constituents can be broadly divided into alkaloids, diterpenoids, steroids, flavanoids and lignans. Reviews have appeared on quaternary alkaloids and biotherapeutic diterpene glucosides of Tinospora species. Much of the work has been carried out on berberine, jatrorrhizine, tinosporaside and columbin. Extracts of *Tinospora cordifolia* (TC) has been shown to inhibit the lipid peroxidation and superoxide and hydroxyl radicals *in vitro*. The extract was also found to reduce the toxic side effects of cyclophosphamide (25 mg/kg, 10 days) in mice hematological system by the free radical formation as seen from total white cell count, bone marrow cellularity and α-esterase positive cells [38]. Extracts of *Tinospora cordifolia* have been shown to inhibit the lipid peroxidation and superoxide and hydroxyl radicals *in vitro*. The extract was also found to reduce the toxic side effects of cyclophosphamide administration in mice. Moreover, administration of the extract partially reduced the elevated lipid peroxides in serum and liver as well as alkaline phosphatase and glutamine pyruvate transaminase. Use of Tinospora extracts in reducing the chemotoxicity induced by free radical forming chemical is widespread [39].

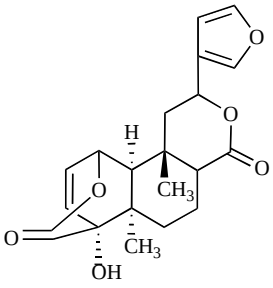
Active principles of TC include Tinosporaside **(3)**, Berberine **(2)**, Columbin **(4)**, Cordiofoliside A **(5)**, Cordifoliside B **(6)**, Cordifolioside C **(7)** and Cordioside **(8)**. These structures provide interesting bioactive scaffolds because of several interesting activities especially related to immune modulation**.** TC was reported to possess anti-complementary and immunomodulatory activities [40] and also for its various immunopharmacological activities such as inhibition of C3-convertase of the classical complement pathway. Humoral and cell-mediated immunity were reported for cardioside, cardifolioside A and cardiol and its activation was more pronounced with increasing incubation time [41]. Extracts of *Tinospora cordifolia* has been shown to inhibit the lipid peroxidation and superoxide and hydroxyl radicals *in vitro*. The extract was also found to reduce the toxic side effects of cyclophosphamide (25 mg/kg, 10 days) in mice hematological system by the free radical formation as seen from total white cell count, bone marrow cellularity and α-esterase positive cells. In a recent clinical study, TC was found to protect against choloroqunine induced spleenomegaly in slow responders, where 35-50% regression of spleen size coupled with increase in Hb was observed suggesting chemoprotection [42] Tinospora bakis: Dose dependent cytoprotection by *Tinospora bakis*; a plant from Senegalese pharmacopoeia was observed *in vitro* model. Lyophilized aqueous extract of plant roots decreased intracellular enzyme release (LDH and ASAT) from CCL_4-intoxicated hepatocytes isolated from rats. Cytoprotective effect was more effective for long course treatment [43].



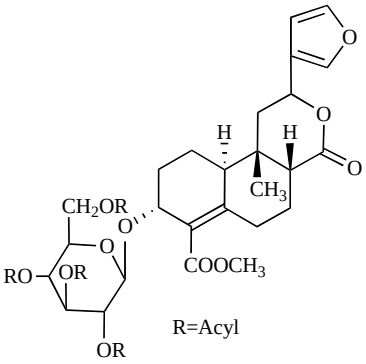
Berberine (**2**)



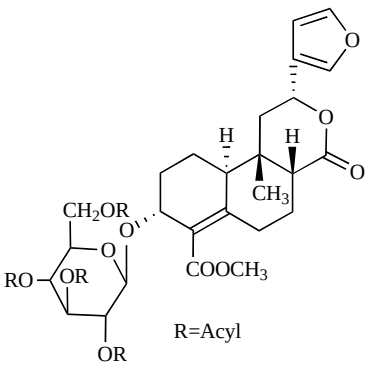
Tinosporaside (**3**)



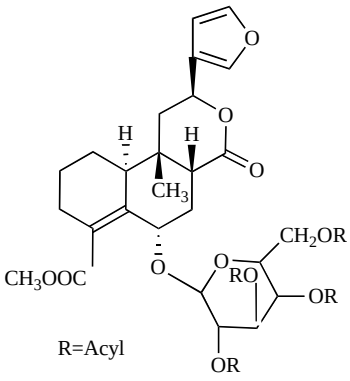
Columbin (**4**)



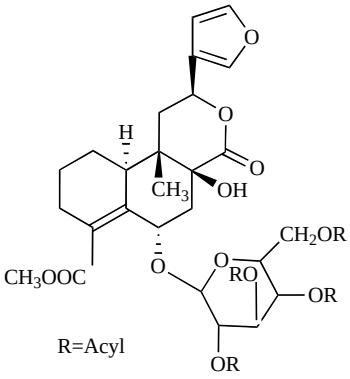
Cordifoliside A (**5**)



Cordifoliside B (**6**)

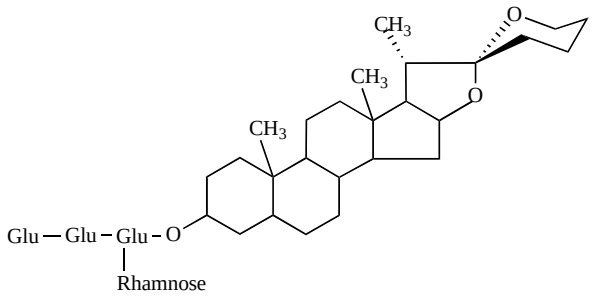


Cordifolioside C (**7**)



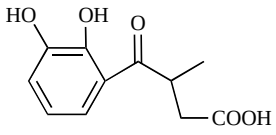
Cordioside (**8**)

Asparagus racemosus: Chemically, *Asparagus racemosus* (AR) contains steroidal saponins, known as Shatavarins **(9),** isoflavaones, isoflavones including 8-methoxy-5, 6,4'-trihydroxyisoflavone 7-O-beta-D-glucopyranoside, asparagamine, a polycyclic alkaloid, racemosol, a cyclic hydrocarbon (9,10-dihydrophenanthrene), polysaccharides and mucilage. It has been shown to stimulate macrophages and influence favorably long term adaptation. Possible links between immunomodulatory and neuropharmacological activity have been suggested. Extracts of *Asparagus racemosus* were evaluated for neuroendocrine immune modulating effect. It prevents stress-induced increase in plasma cortisol along with activation of peritoneal macrophages and inhibition in gastric vascular damage [44]. A comparative study between Asparagus racemosus, Tino-spora cordifolia, glucan and lithium carbonate against the myelosuppressive effects of single (200 mg/kg, subcut.) and multiple doses (three doses, 30 mg/kg, i.p.) of cyclophosphamide in mice revealed all four drugs prevented, to varying degrees, leucopenia produced by cyclophosphamide [45]. Treatment with *Asparagus racemosus* significantly inhibited Ochratoxin A induced suppression of chemotactic activity and production of IL-1 and TNF-alpha by macro-phages [46].

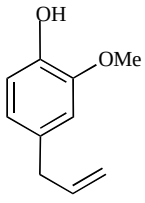


Shatavarin (**9**)

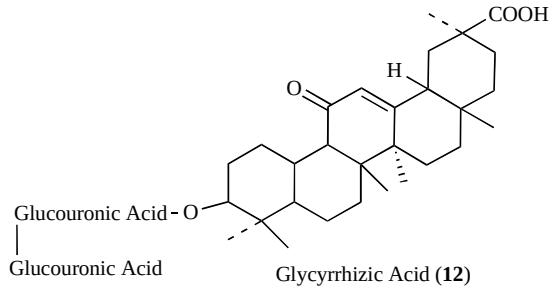
A number of phytochemicals like Caffeine, Genistein, Melatonin, Silymarin, Squalene, Plumbagin (**10**), Eugenol (**11**) and Glycyrrhizic acid (**12**) have multiple physiological effects as well as antioxidant activity which results in cytoprotection. Many antioxidants have additional immunomodulatory and antimutagenic properties and their modulation of cytotoxicity needs further examination and evaluation [47].



Plumbagin (**10**)



Eugenol (**11**)



Glycyrrhizic Acid (**12**)

Picrorrhiza Kurroa (Kutki)

Late Vaidya Zandu Bhatt has popularized Arogyawardhani which is a traditional Ayurvedic formulation for the treatment of jaundice [48]. *Picrorrhiza kurroa* or Kutaki is main ingredient of Arogyavardhini. A double-blind trial with Arogyawardhani, Kutki and placebo was conducted for treatment of viral hepatitis [49]. Significant hepatoprotective effects were observed both for Arogyawardhani and Kutaki. Later, picrosides, the active principles of Kutki were also tested *in vivo* and *in vitro*. Significant antioxidant as well as hydrocholeritic effects were noted for Kutiki [50]. These effects of hepatoprotection in CCl_4 and galactosamine models are considered the pharmacological correlates of clinical actions. However, there is a need to study, at the cellular and molecular levels, how the water outflow in the biliary microcanaliculae is enhanced. A cucurbitacin-free extract of P. kurroa (Picroliv), which is now in Phase III trials [51]. Thus P. kurroa is a good example where the traditional use, observational and experiential data together led to product supported by reasonable evidence base [52]. Various structures including family of picrosides could serve as good scaffolds for fast track drug design, discovery and development of novel hepatoprotectives [53]. A series of preclinical and clinical studies including Phase III trial have established initial safety and efficacy proof. It may be feasible to get new leads in much shorter time as compared to routine target to lead steps in the usual discovery process.

Commiphora Wightii (Guggulu)

Guggulu is a major Ayurvedic drug used widely and in diverse formulations. A monograph of all the major citations has been published. Hypolipidemic effects of guggulu were primarily discovered through reverse pharmacology mode. Antarkar, Satyavati, and Nityanand have extensively studied on the hypolipidemic effect of guggulu [54]. However, the anti-arthritic effects of guggulu in clinic have been relatively less investigated [55]. A sizeable experiential and reverse pharmacological studies with standardized guggulu preparations have been conducted by one of the author (ABDV). Phase I study, long-term and large dose ambulant studies have been conducted. The on-going studies at cellular and molecular levels have helped in evolving pharmacological correlates of clinically shown actions. The side-effects of guggulu have also been documented. Guggulu can offer a platform for technological innovations in pharmaceutics, pharmacodynamics and pharmacokinetics. Topical formulations of guggulu demand unique dermato-pharmacological reverse correlates. Claims, in the literature, on different properties of fresh and old guggulu need to be validated by additional clinical investigations both experiential and

exploratory. Use of such information based on Ayurvedic therapeutics in context of the modern medicine will give many more insights and newer targets in the disease pathogenesis and mechanisms of the drug action [56].

Concept of reverse pharmacology was formally adopted for the fast track development of standardized herbal formulations under an initiative of the Council for Scientific and Industrial Research (CSIR), India. Under the national network project known as New Millennium Indian Technology Leadership Initiative (NMITLI) systematic herbal drug development projects were undertaken. Randomized controlled clinical trials on standardized poly herbal formulations were attempted for treatment of diseases like diabetes, rheumatoid and osteoarthritis and hepatoprotectives with reasonably demonstrated clinical efficacy. The novelty of such research remained in the integrative approach that encompasses reverse pharmacology, ethno medicine, herbal technology and systems-biology approach. The standard elements of a global herbal product-dossier are being addressed. The Ayurvedic knowledge database allows drug researchers to start from a well-tested and safe botanical material. Globally, there is a positive trend towards holistic health, integrative sciences, systems biology approaches in drug discovery and therapeutics that has remained one of the unique features of Ayurveda. A golden triangle consisting of Ayurveda, modern medicine and science will converge to form a real discovery engine that can result in newer, safer, cheaper and effective therapies.

CASE STUDIES: DRUG DEVELOPMENT

NMITLI Osteoarthritis Project

Research partners for this project include Interdisciplinary School of Health Sciences, University of Pune, Pune; Indian Institute of Integrated Medicine, Jammu, (Formerly known as RRL–J); National Botanical Research Institute, Lucknow; Agharkar Research Institute and Interactive Research School for Health Affairs, Pune; Swami Prakashananda Ayurveda Research Center and KEM Hospital, Mumbai; Nizam Institute of Medical Sciences, Hyderabad; All India Institute of Medical Sciences, New Delhi and Center for Rheumatic Diseases, Pune. The project involved industry partners including Arya Vaidya Shala Kottakal; Arya Vaidya Pharmacy, Coimbatore; Dabur, Nicholas Piramal; Natural Remedies, Bangalore and Zandu Pharmaceuticals.

Following prior art [57, 58] and several rounds of national level consultations with Ayurvedic physicians and scholars, a few drugs were short listed. These drugs entered a parallel track of open label observational studies by selected medical practitioners and scientists undertaking animal pharmacology studies. This led to two platforms of drug formulations. This was followed by a randomized, placebo controlled, multi-centric clinical trial where five formulations were put to test with glucosamine as positive control [59]. Two formulations, which performed statisti-cally better than Placebo and Glucosamine were taken up for further studies.

Quality, Safety, Activity and Mechanisms: The quality of crude drugs, extracts and formulations was monitored using Chemistry, Manufacturing and Control (CMC) guidelines of the US FDA. *In vitro* studies using suitable cell and tissue culture models on these formulations revealed significant chondroprotection (proteoglycan release, nitric oxide release, aggrecan release and hyaluronidase inhibition as markers) in explant model of OA cartilage damage. In animal pharmacology, these formulations demonstrated moderate analgesic and anti-inflammatory activities in both acute and chronic models. There was reasonable evidence for synergistic activity in compound formulation as compared to single drugs [60]. The formulations were found to be safe as per OECD guidelines and were devoid of any significant genotoxicity or mutagenic activity in a micronucleus test.

Exploratory Studies: A systematic dosing study was undertaken on the best formulation and the entire data including observational, exploratory clinical trials, dosing studies, *in vitro* and animal pharmacology was subjected to detailed discussions, assessment and analysis by experts. The team realized a need to further augment the analgesic effect, especially for use in osteoarthritis patients. One more ingredient was added to the best formulation. Thus two formulations (one with three ingredients and another with four ingredients) were finalized and taken for manufacture for the final randomized clinical trial of equivalence.

We adopted a platform approach where base formulation based on traditional knowledge was used followed by optimization by adding other ingredients to obtain synergistic activity. All the formulations prepared for clinical trials were manufactured and labeled in accordance with US FDA "Guidance to Industry for botanical drugs". Most of the required tests were performed, during the entire process, starting from passport data of raw material, botanical identification (pharmacognosy), chemical quality (spectroscopic & chromatographic) and molecular (DNA fingerprinting) standardization to stability and pharmacopoeial standards of finished product. Necessary documentation was maintained for review, records or regulatory needs.

All extracts used in formulations were standardized with respect to their marker compounds. Shunthi (Zingiber officinale) hydro-alcoholic extract was standardized with respect to 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol and total gingerols. Salai guggul (Boswellia serrata)hydro-alcoholic extract was standardized with respect to total boswellic acid percentage, boswellic acid (alpha+beta), alpha-boswellic acid, beta- boswellic acid, alpha-acetyl-boswellic acid, beta-acetyl- boswellic acid, 11-keto-beta – boswellic acid, acetyl-11-keto-beta- boswellic acid and acetyl-boswellic acid (alpha+beta). Amla (Phyllanthus emblica) extract was standardized with respect to total tannin as tannic acid and gallic acid. Guduchi (Tinospora cordifolia) extract was standardized with respect to marker tinosporoside.

Clinical Studies: We did exploratory studies on small number of patients in open label model. The observations and experiences of this study were used to optimize study design and test formulations. We then conducted multicentric randomized controlled trial to study five variants of test formulations. The Phase 3 clinical trial was initiated in January 2006 and involved four reputed centers, which followed common protocol. This 24 weeks, randomized,

clinical trial of equivalence with Glucosamine and Celecoxib for comparison, involved 300 patients and is statistically sufficiently powered. The evaluation parameters include clinical assessment as per ACR criteria and most of the critical markers (Urinary CTX-II, Serum Cytokines (IL-1β, IL-6 and TNF-α), Serum MMP-1 and 3, Serum Hyaluronic Acid) are measured in laboratory. Knee X-rays have been taken for 160 patients at baseline and at the completion using a validated digitized image analysis system to measure the changes in the width of the femorotibial joint space, as a surrogate marker for cartilage. At the end of five years a dossier for IND and NDA submissions has been compiled [61]. One Indian one PCT Patent has been filed and currently attempts are underway to transfer the knowhow to suitable industry for further development, optimization, manufacturing and marketing [62].

While we continue to use traditional knowledge and materials in the process of drug discovery and development, its intellectual rights protection has become an important issue. The Government of India has established a Traditional Knowledge Digital Library (TKDL) on traditional medicinal plants and which will also lead to a Traditional Knowledge Resource Classification (TKRC). Linking this to internationally accepted International Patent Classification (IPC) System will mean building the bridge between the knowledge contained in an old Sanskrit Shloka and the computer screen of a patent examiner in Washington! This will eliminate the problem of the grant of wrong patents since the Indian rights to that knowledge will be known to the examiner. It could integrate widely scattered and distributed references on the traditional knowledge systems of the developing world in retrievable form. This will give a major impetus to modern research in the developing world.

FUTURE PERSPECTIVES

In short, for several reasons, the modern drug discovery processes have started revisiting traditional knowledge and ethnopharmacology to reduce the typical innovation deficit faced today that would help reaching to the top in Sciences especially for developing counties like India [63]. A recent analysis article on curcumin published by Cell is good indication of the growing interest in mainstream high impact Journals [64]. An ambitious innovative project to study some of the important basic principles and practices of Ayurveda using most advanced tools of science has been conceived under the name 'Science Initiatives in Ayurveda'. This program is being supported by the, Government of India and involves premier national institutes such as IITs, IISc, TIFR and including Pune and Banaras Hindu Universities [65]. Traditional knowledge and experiential database can provide new functional leads to reduce time, money and toxicity - the three main hurdles in the drug development. These records are particularly valuable since effectively these medicines have been tested for thousands years on people [66]. With Ayurveda, the normal drug discovery course of 'Laboratory to Clinics' actually becomes from 'Clinics to Laboratories'— a true Reverse Pharmacology Approach. It will be in the interest of pharmaceutical companies, researchers and ultimately the global community to respect the traditions and build on their knowledge and experiential wisdom [67]. Systems biology and Reverse Pharmacology approaches need to be developed further and optimized as novel means for fast track drug discovery and development where the newer, safer and effective drugs will remain just a spin off and research continues keeping our hope for block busters alive.

ACKNOWLEDGEMENTS

Although, Reverse pharmacology as a concept was proposed and mainly promoted by one of us (ADBV), it draws inspiration from several researchers who have contributed to evidence based studies on Ayurveda and traditional medicine. We have cited few such scientists and representative case studies, however, there many such who may not have appeared in this article due to limitations on space, we wish to gratefully acknowledge all of them and the great wisdom of several unsung Ayurvedic vaidyas from whom we have received inspirations. We also thank CSIR NMITLI program for formally adopting RP as a strategy for fast track drug discovery and development.

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