1. INTRODUCTION

Herbs are increasingly used for their health and therapeutic benefits globally. More than 50% of the modern drugs have been discovered from herbs & natural products and over 100 new products are in clinical development, particularly as anti-cancer and anti-infective agents\(^1\). Various approaches have been used for discovering newer drugs from herbals (Schematically represented in Figure 1). It includes herbal drug discovery and development from traditional knowledge, modern reductionists and reverse pharmacology (clinics to laboratory) approach\(^2\). There are reports which also suggest the discovery of drugs from herbs by serendipity. Overall herbal drug (HD) development involves several processes starting from generation of passport data on raw materials, processing of raw material into suitable extracts, pre-formulation studies including physicochemical characterization of processed extracts relevant to solid dosage forms, toxicological and pharmacological screening in suitable animal model, and then formulation to suitable dosage forms and evaluation of their therapeutic efficacy using controlled clinical trials.

Although herbal medicines are used worldwide for prevention, treatment and management of diseases, the standardization, quality control and regulation is still a big challenge. There are several bottlenecks in HD development which mainly includes; 1. lack of quality control & standardization checks at various stages of development, 2. lack of physicochemical stability testing of raw materials, processed extracts and their final formulations, 3. Absence and/or insufficient data of pharmacokinetic profiles on bioactive/s after administration of processed extracts to animal models suitable for design and development of dosage form and their regimens, and 4. lack of awareness on possible herb-drug or drug-herb interaction studies (Figure 2). These challenges are due to complex physico-chemical nature of HDs, absence of commercial availability of bioactive marker compounds, lack of sensitive analytical techniques and non uniformity in international regulatory guidelines on HDs. Therefore, there is a need for newer approaches and strategies to overcome such challenges in HD development. Such approaches could help in safe and effective delivery of HDs for health and therapeutic benefits of mankind. In the present research work, an attempt has been made in this direction to address major issues such as quality control, pharmacokinetics and herb-drug interactions using \textit{Gymnema sylvestre}, an important Indian traditional herbal medicine, as a case example.
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Figure 1. Schematic representation of various approaches used for discovery of drug (either single chemical entity or standardized single extract or multi-extract components) from herbs. It involves classical or traditional approach which is based on the use of traditional knowledge from ancient classical texts for the development of herbal drug. In this, raw material is selected (right species and parts) and collected (from appropriate geographical source and harvesting time) on the basis of traditional knowledge. The selected test material first evaluated for safety and then formulated using knowledge of classical formulations (e.g. asavas, aristhas, tinctures etc.). The developed formulations are then studied in controlled clinical trials. Modern HD development is based on reductionists approach, use modern scientific knowledge, highly sophisticated extraction and analytical tools. In this, raw material is processed into suitable extracts (use of non polar and polar solvents). The processed extracts are studied for their toxicity and then bioactivity guided fractionation (use of either successive fractionation with petroleum ether, chloroform, n-butanol and methanol or water etc. or use of flash/preparative chromatography) for intended biological activity (e.g. for cancer, diabetes, etc.) using high throughput screening (HTS, based on cell or enzymes assays). Lead fraction is further purified to lead molecule/s identification. Structural (UV, FTIR, Mass and NMR) and physicochemical (solubility, pH, pKa, etc.) characterization is carried out. Further, pharmacokinetics and bioavailability (absolute) data is also generated for safe and effective delivery of the molecule. Novel delivery systems such as development of nanoformulations, liposomes, phytosomes and ethosomes etc. have been established. Chemical modification, formation of salts and co-administration with bioenhancer has been used as other strategies to improve the oral bioavailability and the efficacy of targeted molecule. The developed formulations are then evaluated further for clinical efficacy. Reverse pharmacology approach is based on experiential science and involves three main stages: 1. experiential phase of robust documentation of clinical observations of the biodynamic effects of standardized Ayurvedic drugs by meticulous record keeping. 2. exploratory studies for tolerability, drug-interactions, and paraclinical studies in relevant in vitro and in vivo models to evaluate the target-activity. 3. experimental studies, basic and clinical, to identify and validate the reverse pharmacological correlates of Ayurvedic drug safety and efficacy.
1.1 Quality control and standardization of HDs: Marker and Fingerprint based approach

Despite of increase in the use of HDs, quality control and standardization remains one of the issues in HD development. Several qualitative tests such as morphology, macroscopy and microscopy have been used routinely for monitoring the quality of raw materials along with processed extracts during HD development. Chemical evaluation of marker compounds and total fingerprint analysis are considered quantitative and semi-quantitative measures respectively for monitoring the quality of several HDs. Marker compounds based approach suggest identification and quantitative measurement of known marker compounds in raw material, processed extracts and finished products using chromatographic techniques like high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC) and gas chromatography (GC). In fingerprint analysis, raw material or processed extracts are considered as drug in its entirety. Chromatographic fingerprint with known and unknown components can be developed, validated and used as quality control tool for raw, processed and finished products. Several hyphenated techniques such as HPLC coupled with diode array detector (DAD) along
with mass spectrometry (HPLC-DAD-MS), GC-MS and HPLC-MS-NMR are reported for online identification of unknown compounds in the developed chromatographic fingerprints. Various chemometric approaches such as similarity analysis, hierarchical clustering analysis, and principal component analysis have been reported for analysis of fingerprint. Several international regulatory agencies such as World Health Organization (WHO), European Medicine Agency (EMA), United States Food and Drug Administration (USFDA) recommends such approaches in their dossiers\textsuperscript{5,6}.

1.2 Pharmacokinetics of HDs: Bioactives and plasma phytopharmacology approach

The fate of herbal drugs especially the standardized extracts or the isolated active phytoconstituents from the extracts after their oral administration has not been explored much yet. It has been well documented that HDs contains either water soluble or lipid soluble phyto-constituents such as terpenoids, flavonoids, tannins, steroids, alkaloids, lipids etc. and are poorly absorbed because of their large molecular size, and poor lipid/water solubility. Phyto-constituents are also known for their gastrointestinal degradation and rapid metabolism\textsuperscript{7}. Pharmacokinetic parameters, such as absorption, distribution, metabolism and excretion (ADME) of phytoconstituents after oral administration of extracts or isolated compounds provides valuable information on bioavailability in plasma and/or targeted tissues and therefore, clinical utility of HDs. Approaches such as bioactive(s) estimation in plasma/or tissues and plasma phytopharmacology has been used to study HD pharmacokinetics. Plasma phytopharmacology is a recently used approach and is based on the identification of phytoconstituents or their metabolites in plasma samples after administration of extracts/or isolated pure compounds thereof\textsuperscript{8}. This approach provides global metabolite profiling in plasma after oral administration of extracts/isolated compounds. Recently, few HDs from traditional Chinese herbal medicines have been studied using plasma phytopharmacology approach and provide useful information on bioactive(s) compounds and their biotransformation in body\textsuperscript{9}. These studies also provide clues on prodrug(s) and identification of prodrug(s) after administration of extract(s)/isolated pure compounds.

Bioactive estimation approach is based on analysis of targeted compounds after administration of extract/isolated compounds\textsuperscript{10}. There are reports available on
pharmacokinetics of curcumin, boswellic acid, glycyrrhizin, ginsenosides after administration of *Curcuma longa, Boswellia serrata, Glycyrrhiza glabra* and *Panax ginseng* respectively\(^{11}\). Interestingly, few reports showed absence of bioactive(s) after oral administration of extracts e.g. oral administration of 2.2 gm (equivalent of 180 mg of curcumin) of Curcuma extract for 4 months daily in cancer patients did not show any traces of curcumin in plasma but showed presence of sulphates and glucuronide conjugates\(^{12}\). This suggested a very low oral bioavailability of free curcumin and need strategies to improve the same. Further, the examples have demonstrated that glycyrrhizin is absent in plasma samples after administration of *Glycyrrhiza glabra* extract and showed the presence of hydrolyzed product, glycyrrhetic acid\(^{13}\). This suggested various pharmacological activities of *Glycyrrhiza glabra* are due to glycyrrhetic acid and not because of glycyrrhizin, thereby suggesting that bioactives could act as prodrugs. Therefore, pharmacokinetics data on bioactives provide useful information on absolute or relative bioavailability as well as on active biotransformed product. Thus, suggesting the strategies to improve bioavailability through various means of designing novel deliveries or synthesis of soluble salts or chemical modification or co-administration with bioenhancers during the herbal drug development phase.

### 1.3 Herb-drug and/or drug-herb interactions: *In silico, in vitro and in vivo* approach

There is increase in prevalence of chronic diseases such as cancer, diabetes, hypertension, heart failure, depression and arthritis in the world\(^ {14}\). Data from 2005 studies conducted in United States of America (USA) showed that 44% of all Americans have at least one chronic condition and 13% have three or more. By 2020, 157 million US citizens are predicted to have more than one chronic disorder, with 81 million having multiple chronic conditions\(^ {15,16}\). In India, the overall projections suggested that chronic diseases would account for about three-quarters of all deaths by 2030\(^ {17}\). Chronic disease conditions such as diabetes, cancer and arthritis etc. are associated with multifactorial pathogenicity, demand multi-modal therapeutic approach. Among multimodal approaches, the use of HDs alone or along with conventional drug is considered as an attractive approach for the treatment of such conditions\(^ {18}\). HDs are taken by the patients with a belief that they are safe and helpful in reducing side effects associated with the conventional drugs. They are generally
taken with or without concern with physician. It has been well documented that as many as 31% of patients use herbal drugs concurrently with conventional drugs and 70% of them do not report the use of these products to their physician. The use of such combination direct to pharmacokinetic and/or pharmacodynamic interactions with conventional drugs and leading to beneficial, sub-therapeutic and/or toxic effects. Such combination therapies need to be tested for their herb-herb, herb-drug and drug-herb interactions in early phase of herbal drug development. Several approaches such as use of in silico, in vitro tissue systems and in vivo models have been suggested for the evaluation of herb-drug interactions during the drug development. In silico approach with the use of pharmacophore model as well as the docking technology for the early prediction of drug interactions. Induction and inhibition of drug metabolizing enzymes and transporter proteins by HDs has been documented in various in vitro studies for the prediction of possible drug interactions. Recently, USFDA guidance on drug interaction studies recommended such in vitro assays based on human microsomes, cytochromes and hepatocytes and also suggested the use of positive assay control (for cytochrome P450) during the development of in vivo studies for HDs. While these studies offer a system to determine the potential for a herbal component to alter the pharmacokinetics of a drug, they cannot always be used to predict the magnitude of any potential effect in vivo. In vivo studies are the ultimate way to determine the clinical importance of herb-drug interactions. Traditional Chinese herbal medicines (TCHM), Western herbal medicines, Kampo, Cuba and African herbal medicines have been reported for their herb-drug interaction studies with conventional drugs used in several chronic conditions. Allium sativum (Garlic), Gingko biloba, Ginseng (Panax ginseng), Glycyrrhiza glabra (Licorice), Piper methysticum (Kava), Silybum marianum (Milk Thistle) and Hypericum perforatum (St. John’s wort) have been reported as the most studied herbs for their pharmacokinetic as well as pharmacodynamic interactions with drugs used in chronic conditions such as cancer, cardiovascular diseases, nervous disorders, diabetes and arthritis etc. Documentation and reporting of such studies is of vital importance to physician, patients and pharmacists for rationalization of the combination therapy and to avoid adverse event associated with interactions. Evidence based research on risk versus benefit assessment of concomitant use of herbs with conventional drugs is lacking. Several international regulatory agencies such as WHO, USFDA, EMA and Australian Regulatory Guidelines for
Complementary Medicines etc. recommend and advice such studies during herbal drug development.

1.4 Scope of proposed investigation: Gymnema sylvestre as case example

*Gymnema sylvestre* (commonly named as Gurmar, family: Asclepiadaceae) is one of the well known plant used in diabetic conditions and is officially mentioned in Indian Pharmacopoeia (Indian Pharmacopoeia, 2007). Traditionally, it has also been used as stomachic, remedy for cough and anti-diuretic. *G. sylvestre* is the one of the major ingredient of various single as well as multi-herb formulations used for diabetic conditions globally. Several preclinical studies on polar/non polar extract of roots and leaves of *G. sylvestre* suggested anti-hyperglycemic, anti-hyperlipidemic, anti-microbial, anti-oxidant, anti-inflammatory and anti-cancer activities. Mechanistic studies suggested that *G. sylvestre* exert its hypoglycemic effects through increase in insulin secretion, regeneration of islets cells, peripheral utilization of glucose, and inhibition of glucose absorption from intestine. Further, clinical reports validate the use of *G. sylvestre* in Type-1 and Type-2 diabetic conditions.

Chemically, gymnemic acids are acidic glycosides isolated from the leaves of *G. sylvestre* and have been reported as anti-sweet compounds, or sweetness inhibitors. The total saponin fraction, referred to as gymnemic acids, of the leaves of *G. sylvestre* has been identified as the active fraction, and the quality of *G. sylvestre* extracts and formulations are assessed by the content of gymnemic acids. Several methods, including HPTLC, HPLC and HPLC coupled with mass spectrometry, are available for quantitative and qualitative determination of gymnemic acids, directly or indirectly. Direct estimation of gymnemic acids is very difficult because they are complex mixture of several closely related compounds and are not available commercially as reference compounds. An indirect method for quantitative analysis of gymnemic acids using HPTLC has been reported for quality control on the basis of a hydrolyzed product known as gymnemagenin (GMG). The existing methods have limitations such as lack of sensitivity, requirement for a large amount of sample and a derivatization process for detection, as well as low sample throughput. Therefore, there was a need for a rapid, sensitive and selective method for quantitative estimation of GMG in *G. sylvestre* samples. High performance liquid chromatography/tandem mass spectrometry (HPLC–MS/MS) has been used successfully for identification and quantitation of several compounds in complex samples, such as plant extracts and
biological fluids\textsuperscript{40, 41, 42}. The HPLC–MS/MS technique provides specific, selective and sensitive quantitative results, often with reduced sample preparation. Despite of the extensive phytochemical investigations and mechanistic studies on \textit{G. sylvestre} extract, as prerequisite, there is a need of more accurate, sensitive and selective analytical tool for quality control and for monitoring its concentration in plasma for pharmacokinetic evaluation. Pharmacokinetics data can provide valuable information to aid practitioners in prescribing herbal drugs safely and effectively. Therefore, in the present research, an attempt has been made to develop more accurate, sensitive and selective analytical tool for quality control, standardization and monitoring its concentration in plasma for pharmacokinetic evaluation of \textit{Gymnema sylvestre} extract and its nanoformulation. A cursory review of literature available had also indicated that there is no direct published report on the anti-diabetic and anti-hyperlipidaemic activity of GMG. Therefore, in the present research, an attempt has been made to identify the fate of GMG, its water soluble salts and nanoparticles in the diseased (diabetic) biological system. Further, \textit{Gymnema sylvestre} extract when used with oral hypoglycemics, as generally people do, may lead to drug interactions. Therefore, the present work also been undertaken to address the herb-drug interactions using \textit{in silico} and pharmacokinetics & pharmacodynamics approach in diabetic animals on an important Indian traditional medicine, \textit{Gymnema sylvestre} \textit{R.Br.}, as a case example.