Pharmacodynamic Evaluation: Herbal Medicine

Gulam Mohammed Husain, Mohammad Ahmed Khan, Mohd Urooj, and Munawwar Husain Kazmi

Abstract

Herbal medicines are the primary therapy available to large segment of population across the globe. Globalization of herbal medicines has expanded their market to industrialized countries. Regulatory agencies have also extended their attention towards herbal medicines, although the majority of markets still remain largely unregulated. Further, important clinical information related to efficacy, effectiveness, dosage, adverse effects, and contraindications of herbal medicine needs to be generated to bring them into the mainstream healthcare. Research into molecular effects and clinical efficacy of the numerous herbs are ongoing. Pharmacodynamic evaluation of herbal medicine through clinical studies can provide scientific evidence on benefit/risk against a particular disease. However, there are numerous challenges in conducting efficacy studies on herbal medicines. The conduct of placebo controlled clinical trials of herbal medicine poses ethical issues. It is difficult and sometimes not feasible to mimic the organoleptic properties of herbal medicines while using placebo arm. Randomization of trial subjects may be against the principles of practice of systems of medicines based on holistic approach of treatment. Varying doses mentioned in literature also makes it difficult to optimize clinical dose for testing in humans. Lack of stringent quality control of formulation aspects can lead to varying outcomes in clinical studies. This chapter discusses the ethical aspects, design consideration, quality issue and pharmacokinetic and bioanalytical challenges in pharmacodynamic evaluation of herbal medicines in clinical setting.

Contents

G.M. Husain (✉) • M.A. Khan • M. Urooj
Pharmacology Research Laboratory, Central Research Institute of Unani Medicine (Under CCRUM), Hyderabad, India
e-mail: gmhusain@gmail.com; khan.ahmed1511@gmail.com; qaziurooj@gmail.com

M.H. Kazmi
Department of Ilmul Advia (Unani Pharmacology), Central Research Institute of Unani Medicine (Under CCRUM), Hyderabad, India
e-mail: drmunawwar@yahoo.com

F.J. Hock, M.R. Gralinski (eds.), *Drug Discovery and Evaluation: Methods in Clinical Pharmacology*,
https://doi.org/10.1007/978-3-319-56637-5_52-1

Background

Globalization of Herbal Medicines

Plants and herbs have been used by mankind for ages and are now being used globally for the treatment and prevention of disease. Plants also contribute to a number of clinically used modern drugs comprising of approximately 25% of drugs prescribed globally. However, plants are more commonly consumed as herbs and herbal extracts often under categories of drugs (as herbal medicines and traditional medicines) and food (as dietary supplements) (Xutian et al. 2009; USFDA 2016). As per WHO, herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain, as active ingredients, parts of plants, or other plant materials, or combinations. Traditional herbal medicines (or traditional medicines) are the herbal medicines which have undergone no or minimal processing and have been used as per regional or local healing practices (Tilburt and Kaptchuk 2008). Some herbs having health-promoting effects are also marketed as such in crude form.

Herbal medicines are used both as primary treatment as well as adjunct to conventional drugs by different populations. Especially in some developing countries, traditional system of medicines is the only system of treatment available to a large population. According to WHO, 90% population in Africa, 70% in India are dependent on herbal medicines for their healthcare needs (WHO 2005). Traditional medicine units are present in more than 90% general hospitals of China. Herbal medicine in Germany is part of one of the five main elements of naturopathy (also called as Kneipp therapies). The percentage of german population using herbal medicines increased from 52% in 1970 to 70% in 2010. In 2011, Germany spent one billion Euro on herbal medicines which is approximately 20% of the total expenditure for over the counter (OTC) drugs (Joos et al. 2012).

Recent decades have seen the globalization of herbal medicines leading to expanded use of ethnobotanicals in the industrialized countries. According to National Center for Complementary and Alternative Medicine in the United States, herbal medicines are the most commonly used (18.9%) alternative medicine (Barnes et al. 2008). In two different surveys, 38% of US adults have reported use of herbal medicine whereas 40% of Hong Kong population have shown faith in traditional herbal medicines (Barnes et al. 2008; Ernst et al. 2005; Chan et al. 2003). As of 2008, the global annual turnover of herbal medicines had reached nearly US$ 80 billion (Anonymous 2017) with millions of US dollars of industry investment being made in medicinal herbs with promising potential (Zamiska 2006). The global herbal medicine market was expected to reach $107 billion by the year 2017 (Anonymous 2012). However, this investment still remains small compared to huge size of global pharmaceutical market. National health authorities are also increasing their attention towards herbal medicines due to the excessive reliance of less developed countries on herbal medicines as well as due to expanding base in developed countries.

Irrespective of the reason for their choice, the consumers have right to receive herbal medicines that are safe and effective. Further, scientific studies need to be carried out to generate science based information on efficacy, effectiveness, dosage, adverse effects, and contraindications. Research into molecular effects and clinical efficacy of the numerous herbs are ongoing. Pharmacodynamic evaluation of herbal medicine through clinical studies is an important step of this process which can provide scientific evidence on benefit/risk of any herbal medicine in the treatment of disease.

Regulatory Challenges

The diverse countries practice herbal medicines due to historical reasons as well as different holistic approaches. This makes their regulation a difficult task. Further, regulating large number of herbs is also a challenge. The WHO survey of 129 countries identified lack of research data, herbal medicine control mechanisms, safety monitoring, and methods for evaluation of safety and efficacy as some of the challenges in regulation of herbal medicines. Regulatory authorities of various countries are yet to develop strict guidelines for robust assessment of safety and efficacy.

Notwithstanding, many markets of herbal medicines have remained largely unregulated leading to poor quality control during sourcing of raw materials, production, distribution, and sale. In addition, there exists an overlap in definitions of plant-based products among different countries. The same plant or plant-based product may be defined as a dietary supplement, food, or herbal medicine by different regulators. For example, EMEA defines herbal drugs as the whole, fragmented or cut, plants, parts of plants, algae, fungi, lichen in an unprocessed state usually in dried form or afresh. Herbal drug preparations are the herbal drugs which are subjected to various treatments and prepared as tinctures, extracts, essential oils, expressed juice, or process exudates. In contrary, USFDA defines all herbal products as dietary supplements and therefore as foods. Whereas, USFDA defines a Botanical Drug Product as derived from plants, algae, or macroscopic fungi and prepared from botanical raw materials by one or more of the processes such as pulverization, decoction, expression, aqueous extraction, ethanolic extraction, or other similar process, intended for use as a drug. Although, safety of herbal medicines remains a global concern, a global survey conducted by WHO revealed that till 2003, 63% of 191 member states did not have laws and regulations for herbal medicines and herbal medicines were sold as over-the-counter (OTC) products in 68% of member states. In relation to quality, only 24% countries had national pharmacopoeia of herbal drugs (WHO 2005, 2008).

The registration requirements for herbal drugs also vary from country to country. For example, herbal drugs with sufficient evidence of the medicinal use of the product throughout a period of at least 30 years, including at least 15 years in the community have been exempted from testing and trials for safety and efficacy by the Committee on Herbal Medicinal Products (HMPC) within the European Medicines Agency (EMEA). In India, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) is the main regulator for herbal medicines particularly traditional system products majority of which do not require stringent safety and efficacy studies as governed by The Drugs & Cosmetics Act.

USFDA registers herbal products used in complementary and alternative medicine which, based on its use, may be regulated as food (including food additives and dietary supplements), drug, cosmetic, or biological product. Further, in such case, no exemption in regulation is granted to herbal medicines. For example, a dietary product used for promoting optimal health may be subject to the requirements for foods including all the safety and quality testing as per FDA regulations. Whereas, if the same is to be used as a treatment regimen for a particular disease, it will be subject to regulation as a drug. The product may also be categorized as "new drug" under the Act and premarket review and approval by FDA may be needed which includes evaluation of safety and effectiveness under the prescribed conditions as suggested in the labeling.

Ethical Aspects in Conducting Human Studies

Clinical trial is the foremost part of drug development process involving human subjects. Clinical evaluation of herbal medicine should be subjected to alike ethical principles as per national and international guidelines and regulations implicated for conventional medicine. Some of the prominent code of ethics and set of laws which direct ethical clinical research include Nuremberg Code (1947); Belmont Report (1979); US Common Rule (1991); Declaration of Helsinki (2000); and Council for International Organizations of Medical Sciences (2002).

The major ethical shortcoming of a clinical trial is the population which will gain is not the same which is taking the burden of risk by participation. Thus, all guidelines and regulation are stern to protection of rights, safety, and well-being of clinical trial participants. Clinical trial for herbal medicine must be conducted as per defined protocol approved by ethics committee as well as guidelines of regulatory agency. Below mentioned points in Fig. 1 are ethical concern needed to be addressed for clinical research:

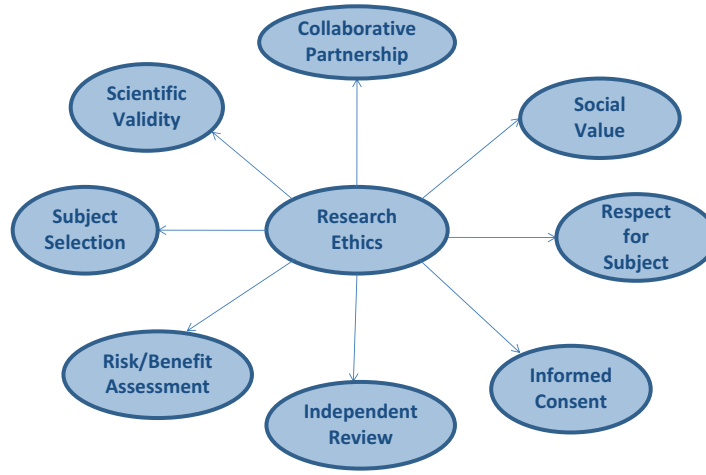

Fig. 1 Ethical consideration for clinical trial on herbal medicine

Risk and benefit ratio of trial drug should be identified and assessed to minimize the risk and maximize the benefits to participant. However, safety of the subjects on herbal product trial is often overlooked, because it is believed that herbal medicine is safe as being used clinically from prolonged time and sometimes from centuries. This may cause error in risk assessment leading to serious consequences. For instance, the whole ephedra plant traditionally used to treat bronchial asthma possess toxicity potential including cardiac arrhythmia, myocardial infarction, and stroke (Koonrungsesomboon and Karbwang 2016).

Another ethical issue is using placebo arm in controlled clinical trial of herbal medicine. Depriving a patient of active treatment is unethical especially in critical disease like diabetes, cancer, etc. It is therefore recommended to conduct an add-on trial for herbal medicines which may be useful to prevent adverse effect of modern treatment until system is not able to standardize and optimize the dose, regimen, and duration of treatment. Herbal medicine trial involving geriatric population also raises an issue of ethics, due to concern of age and impaired organ function which may alter the pharmacokinetics and pharmacodynamic of trial drug. The dose which is considered to be safe in general population may become toxic in elderly population (Routledge et al. 2004). Ethical standards must be kept into account in line with national and international guidelines for herbal drug clinical trials without compromising the rights, safety, and well-being of trial participants.

Design of Clinical Trials for Herbal Medicines

Control and Randomization

Control

A control or control group may be defined as a group of clinical trial participants which do not receive the drug or treatment being investigated as part of the trial. The control group is essential in controlled clinical trial to ascertain the effectiveness of study drug by nullifying the effect of confounding factors. There are four major controls that may be utilized while conducting clinical pharmacodynamic evaluation of a herbal medicine viz. placebo control; active treatment control; no treatment control; and different doses or regimen as control.

The foremost challenge with trial of herbal medicine is selection of appropriate control. Selection of placebo control puts forward the challenge of mimicking the exact taste, color, odor, texture, and physical state as treatment. For example, preparation of placebo for decoction, complex natural product having strong aroma and odor-like ginger or garlic, etc. The second issue is opting for placebo instead of availability

of an active treatment, which is ethically unjustified to let the patient without treatment when standard therapy is available.

Similarly, in order to compare the efficacy of a test drug, an established and widely accepted drug must be chosen as active (positive) control. The active control trial has merit of ethical and practical concern. The primary choice for active control drug selection is that the drug should have indistinguishable mechanism of action or similar action as test drug. If it is not available, then drug of same therapeutic class with same indication has to be chosen. Herbal drugs may be used as an active control wherever feasible such as silymarin for hepatoprotective activity. Selection of active control is also governed by the nature and complexity of the herbal drug, for example, enriched fraction or whole extract; crude preparation or isolated active ingredients.

The selection of active control group from conventional medicine for comparison of efficacy of herbal drugs is debatable as it definitely influences comparability of groups. The outcome for both active control and test treatment could not be equally comparable due to lack of similarity in mechanism of action, dose optimization, variability in dosage regimen, and differences in formulation. Choosing no treatment as control will reduce the power of study and at the same time will be ethically unjustified. This control generally applied in experimentation of animal and clinical trials of chronic disease.

The way forward for placebo development is to move towards redesigning of dosage forms without compromising the holistic approach. The capsule as dosage form might solve the problem for herbal placebo with certain limitations like the formulation of capsule for herbal medicines with large doses. Further, in case of herbal drug clinical trial for traditional medicines, practitioner or government research organization must try to adopt robust research methodology in order to achieve the best possible active control. One possible approach is planning and conducting a pilot study with low sample size, to generate the preliminary data in support of efficacy of herbal drug. A confirmatory trial may further validate the findings in a larger, heterogeneous population.

Randomization

Randomization is a method by which study participants are assigned to a treatment group based on chance alone. Randomization generates comparable group of treatment by eliminating possible source of bias. Randomization ensures that each patient under trial have equal chance to receive any treatment which is achieved by concealment of allocation where neither patient nor researcher is aware about the treatment assignment to trial subject (Suresh 2011).

There are some statistical methods like ANCOVA (analysis of covariance) which are used to adjust imbalance among covariates between trial arms during analysis stage. There may be variation in adjustment required for each trial group, however, the major limitation with ANCOVA is that this method uses the average slope among groups to adjust the outcome variable. Therefore, the best method to achieve balancing among covariates is to apply randomization at the stage of designing of clinical trial instead of applying statistical tool after data collection (Kabisch et al. 2011).

The randomized clinical trial (RCTs) is considered as gold standard for the assessment of treatment effect. RCTs are the highest power clinical designs to study the safety and effectiveness of new treatment or intervention and mandatory for obtaining approval by government regulatory bodies (Bothwell and Podolsky 2016). RCTs are mainly trials that entail one arm as control group where experimental group is compared with control group (see section on control above for different types of control). In eighteenth and early nineteenth century, the trials conducted were poorly controlled to confirm the effectiveness of orthodox wide range medicine. Major development of scientific methodology in late nineteenth and twentieth century raised the demand of rigorously conducted controlled trials to prove safety and effectiveness of new medication.

There have been adequate studies conducted on herbal drugs, but well-controlled clinical trials are still lacking to prove safety and efficacy, for example, the outcome of clinical trial on extract of *Ginkgo biloba* used to treat CNS and cardiovascular disorders and *Hypericum perforatum* (St.

John's wort an antidepressant) prove that both drugs are quite safe and effective. However, their clinical efficacy still needs to be supported by further well-designed controlled clinical trials. The issue of relevance of randomized clinical trial has also been raised by the medical practitioner prescribing herbal medicines since they argue that they are treating soul, mind, and body at the same time with holistic approach. These physicians are integral part of therapy and treat the patients on individual basis and therefore the outcome of the treatment cannot be generalized. Although RCTs on herbal medicines are difficult to execute, but these are the need of the hour. They require a careful planning and a team effort for establishing the efficacy of herbal medicines. Recently, the new approach of partial randomization has been proposed where first the patients are given a choice to decide the trial arm preference and allocated to the arm based on choice followed by randomization of remaining patients who do not opt for any preference. Similarly, according to the design proposed by Harvard Statistician Zelen, patients are first randomized to control or treatment arm prior to seeking informed consent followed by conditional sought of consent. As per Zelen's design, consent may not be sought from the patient receiving standard care except for privacy reasons.

The double screening recruitment model is an alternative recommended for clinical trial on herbal medicine with traditional origin where clinical trial subjects are first screened using modern diagnosis techniques and then categorized based on traditional classification system. Here the patients get equal opportunity to receive experimental treatment or standard treatment. Crossover design is possibly a better choice in case of stable disease and therapy that have short-term effects. Crossover design minimizes the variation as a patient becomes its own control, i.e., in the first phase patient will receive experimental drug and after washout period same patient will receive the placebo. Design adaptive allocation is also a suggested technique over randomization which ensures better balance between measured and unmeasured outcomes. This method also enhances statistical power.

Optimization of Clinical Dose

There are two vital transitions in clinical trials: first transition is from preclinical pharmacology study to first in human use, i.e., phase I clinical trial, and second transition is from phase IIa to IIb/III where large number of patients are exposed to the drug (Gobburu and Gopalakrishnan 2006). Both transition phases have significant influence on selection of dose levels. Selection of suboptimal dose level will impact the project timeline while higher than therapeutic dose level may produce adverse effects. In light of above facts, the major concern for herbal medicine trial is dose optimization as varied clinical doses are mentioned in the literature and there is lack of consensus regarding the dose regimen. The objective to bring herbal medicine parallel to conventional medicine in healthcare system can only be achieved by developing proof of concepts on scientific grounds.

Reverse pharmacology is the technique to prove the experience based claims for traditional herbal agents by applying modern scientific methods. The major challenge while designing clinical trial for herbal medicines is selection of dose levels and regimen. Three scenarios come into play while applying pharmacodynamic principle to evaluate herbal medicines (Parveen et al. 2015).

(a) Herbal agents whose efficacy is demonstrated, active constituent is known, and their doses are more or less established
(b) Herbal agents with putative efficacy which needs to be demonstrated and active principle needs to be standardized
(c) Herbal agents with uncertain efficacy but used traditionally since centuries

The solution to these challenges is rigorous quality control of the herbal drug and standardization using as many as possible analytical markers. Modern analytical techniques such as hyphenated techniques like LC-MS, GC-MS needs to be adopted for standardization of herbal medicine. Formulation of herbal medicine must ensure minimum batch to batch variation.

Application of pharmacodynamic principle is justified only when the identity and composition of the herbal drug is well characterized. Another challenge is pharmacokinetic profiling of herbal drugs whether it is a single or multiple constitutent product. Identification and quantification of the principle biomarker(s) in biological fluids require high level of expertise.

Dose optimization can possibly be achieved either by conducting efficacy study in animals at initial levels and then by translating results for first in human studies or directly conducting phase I trial. Phase I trial must be undertaken to identify maximum tolerated dose and measurement of drug activity. Phase I trial with dose escalation method as mentioned in Fig. 2 can be conducted with low sample size. The trial subjects should be randomly allocated to three different groups with low dose group, middle range dose group, and high dose group. There should be no difference in inclusion criterion for all groups. The safety and efficacy is the primary objective for phase I trial which will be assured by changes in standardized efficacy endpoints at different dose levels. If we are able to find out maximum tolerated dose level and efficacious and safe dose level for these herbal agents then randomized controlled trials could be planned. It must be kept in mind that it is generally claimed that herbal drugs do not cause any adverse effect; however, phase I trial must be carried out under extensive medical supervision as per ICH-GCP guideline without compromising patient safety.

Selection of Study Duration

In conventional medicine dosage regimen is strictly followed to maintain uniformity; however, there is wide variation in dose and treatment duration in case of herbal or traditional medicine. Herbal medicine, either single constituent or polyherbal formulation, are frequently used as self-medication by the patients with varied duration until relieved symptomatically. Similarly, traditional practitioners prescribe same drug for same indication for different duration based on the symptomatic relief. They claim that disease and treatment is individualized to the patients in case of alternative medicine. However, for any new herbal medicine, clinical studies are needed to be designed for optimization of dose, dosage regimen, and treatment duration. This objective can be achieved by designing a pilot study with low sample size, standardized disease specific inclusion-exclusion criteria and clinical efficacy end points. Optimized dose should be given to patients with stringent follow up for uniform duration decided as per available literature and gathered experience. The follow up should be done till the patients become healthy or relieved symptomatically as well as based on the achievement of previously set objective parameters. Relapse of disease must also be kept in mind and duration of follow up may be extended accordingly.

Efficacy and Safety Assessment

Efficacy or effectiveness of herbal drug could be confirmed based on the clinical end points. Clinical end points (efficacy end points) are used to measure the effect of a drug by assessment of clinical events. The end points are classified into three categories viz., primary, secondary, and exploratory. Primary end point becomes main objective of clinical trial for which trial subjects are randomized and study is powered, for instance, chest pain becomes primary end point for trial of drug preventing heart attack. Primary end point is the main outcome of the study to address the research question and main hypothesis.

Secondary end points are additional clinical outcome/events for which neither subjects are randomized nor is the study powered for. Secondary end points do not possess similar statistical authority as primary end points, for example, drug for osteoporosis using fracture as primary end point and improvement in bone density as secondary end point. All other end points recorded in a study are known as exploratory end points (USFDA 2017).

The practitioners of traditional herbal medicines use different methods of diagnosis compared to conventional practitioners. Herbal

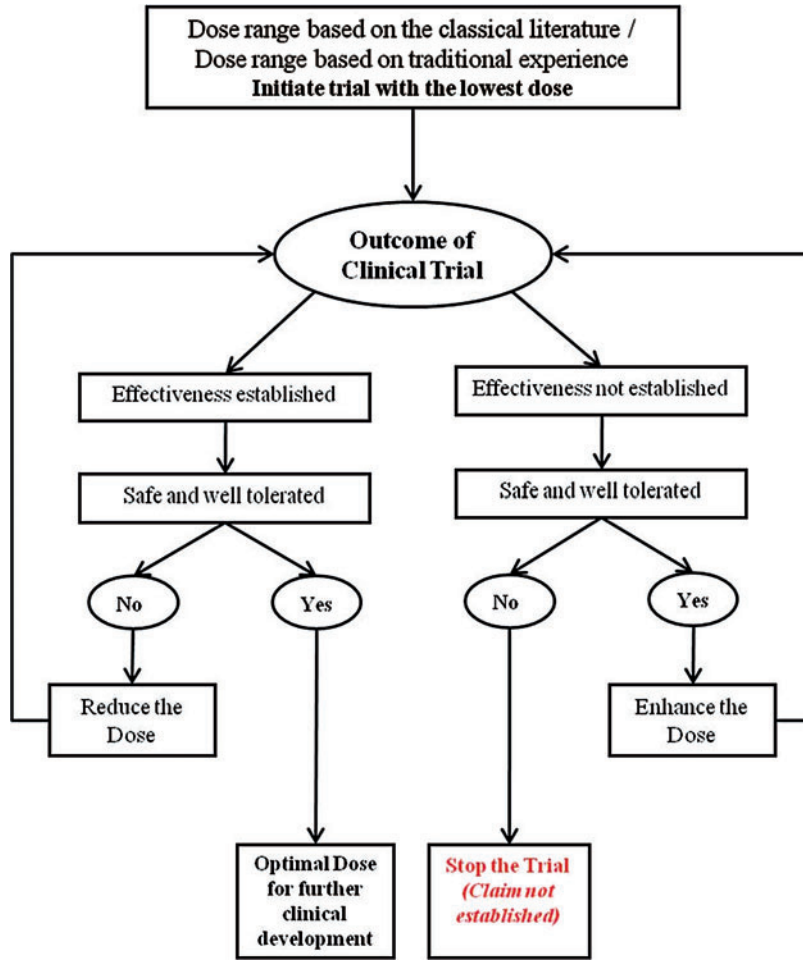

Fig. 2 Dose escalating study

practitioner uses subjective end points generally focused on relieving the symptoms and most of the time relates a disease with under-functioning of a biological system for instance the cause given by traditional herbal practitioners for arthritis is accumulation of waste products in joints for which they prescribe the combination of herbs such as diuretics, choleretics, laxatives, and additional anti-inflammatory herbs (Vickers and Zollman 1999). Conversely, modern diagnosis is based on hard end points which can be accurately measured.

The scenario is rapidly changing now and therefore, randomized controlled trials are needed to be conducted to optimize herbal medicine outcome in line with modern clinical end points. For example, herbal medicines have promising outcome in case of renal and hepatic diseases but there is a need to confirm the results based on the biochemical and molecular evidences of renal and liver function.

Herbal drugs are perceived of being safe and devoid of any side effects because these medicines have long history of use. However, the concept of safety is relative and all medicinal products are associated with some risk in addition to their beneficial effects. The benefit and risks are always addressed with caution prior to marketing authorization of pharmaceutical product. Randomized controlled clinical trials are the best way to provide evidence for safety of medicinal product before marketing approval. There is a need of extensive clinical research to ascertain the safety of herbal medicine by conducting randomized

controlled clinical trials. Further, there are some aspects of safety which could not be addressed by clinical trials hence, prior to clinical studies, non-clinical assays like preclinical carcinogenic study, developmental toxicity study, reproductive toxicity study, and repeated dose toxicity studies must be performed. There is a concern of safety in instances where herbal drugs are to be coadministered with modern medicine or with another herb or nutrients. Therefore, drug interaction studies are recommended in such cases to ensure safety. Considering the huge exposure potential of herbal drugs, researchers must also try to generate the data for post marketing vigilance for safety of herbal drugs.

Chemistry Manufacturing and Control (CMC) of Herbal Medicines

Materials of herbal origin used in herbal medicines are uniquely distinct from conventional modern medicinal products. Unlike conventional pharmaceuticals which are manufactured using synthetic molecules and reproducible processes, ingredients of herbal medicine are obtained from diverse sources. Thus, it becomes difficult to ascertain the past conditions to which these were subjected to. Further, one particular herbal material obtained from more than one cultivation source varies in quality and composition. Moreover, there are seasonal variations in the constituents of plants from the same origin. The heterogenous nature of botanicals also makes it difficult to identify active constituent, ascertain uniform quality and rule out contamination with other plant materials. Due to these reasons, the conventional methodologies cannot be used for manufacture as well as quality control of herbal medicines. Since, the herbal medicinal products are affected by processing and production techniques, quality assurance has become an important prerequisite for conducting clinical trials on herbal medicines.

Standardization and Quality Control

Primary objective of standardization and quality control is to ensure that the product batch/batches are consistent in terms of their composition, strength, and pharmacological effect. Quality control of herbal medicines involves a battery of tests including identification, assay of chemical constituents, inorganic impurities (including toxic metals), microbial load, pesticides, etc. (Ong 2004). "Phytoequivalence" is used to ensure consistency of herbal medicine by comparing the chromatographic fingerprint with the profile of a reference product (Liang et al. 2004). In summary, quality control of herbal medicine requires a totality of evidence approach.

USFDA recommends that the quality control of botanical drugs should extend to raw material (s) and may additionally require biological assays and data on outcome variations from multiple batch clinical study. Following three have been identified by USFDA for quality control of botanical drugs (USFDA 2016):

1. Botanical raw material control
2. Quality control by chemical tests
3. Biological assay

1. *Raw Material*
   Raw material from at least three cultivation sites should be collected for the assessment of quality. The same material should be used as representative materials for evaluating therapeutic consistency and the same should be used for the production of raw material batches for multiple batch phase III studies. There may be need for additional characterization of raw material using spectroscopy/chromatography, DNA fingerprinting, etc.
2. *Herbal Medicinal Product*
   Complete pharmaceutical development is required for herbal medicinal products to avoid any changes in either raw material or manufacturing processes for clinical studies. Post clinical development, robust manufacturing is required to demonstrate that the herbal product to be marketed is equivalent to the one used in clinical study. The key factor in

ensuring consistency as well as quality of batches is the reproducibility of the production process. This requires validation of all process including qualification of equipments and establishment of a formal change control system to identify effects of changes on the quality.

3. *Bioassays*
   While the raw material and drug product control are essential for establishing identity and ensuring quality, in some instances correlations between the quality and biological activity may be required using bioassay. The bioassay can ensure therapeutic consistency. The bioassay should preferably be closely related to the test drug's proposed mechanism of action.

Pharmacokinetic and Bioanalytical Challenges

Herbal medicines have become popular as complementary therapy especially against a number of chronic conditions like cancer and metabolic diseases (Hollander and Mechanick 2008). The mechanism of action of herbal medicine is different from the modern drugs having single chemical moeity. It involves a "network" approach, wherein multiple compounds act together through interaction with multiple *in vivo* targets with interdependent actions leading to optimal effect (Chan 1995). The main objective of carrying out pharmacokinetics (PK) study is to understand the *in vivo* process that a drug undergoes. PK is an integral part of drug discovery process (Wu et al. 2000). Thus, the extensive PK study of herbal medicines can play critical role in improving the understanding of clinical effects and generation of evidence for design of a reasonable dosage regimen. However, due to lack of proper understanding of PK of herbal medicine (especially multicomponent and traditional system medicine), currently the design of dosage regimen of almost all such products is based on ancient empirical therapy rather than *in vivo* profiles. This rationality in clinical practice has not been accepted by the modern medical system. In addition to the design of dosage regiment, PK studies of herbal medicines can also aid in developing better understanding of their interactions with other exogenous systems. Interactions, between phytoconstituents and prescription drugs have recently been in focus, especially due to increasing awareness among medical practitioners about the widespread adverse effects of herbal treatments undisclosed by the patients (Mukherjee et al. 2015). The Committee on Herbal Medicinal Products (HMPC) has mandated that interaction of herbal medicine with other medicinal products (EMEA 2007a, b) must be evaluated. Similarly, FDA guidance for botanical drug products has also described that assessment of interactions between herbal medicines and other commonly used drugs and/or dietary supplements must be carried out (USFDA 2016).

Challenges in the Assessment of Pharmacokinetics

Generally, the pharmacokinetic principles used for development of single molecule drugs have been employed to understand the efficacy and toxicity of herbal medicines. Some of these principles include (Na 2010):

1. Assessment of rate and extent of absorption of components of herbal medicines
2. Understanding of metabolic fate of component (s) of herbal medicines
3. Elucidation of route(s) of elimination and elimination kinetics
4. Understanding herbal medicine-synthetic drug interaction

However, this strategy has not been able to achieve much success because of complexity of extracts especially in case of multicomponent mixtures. The high number and wide range of metabolites present in natural products are inextricable obstacles in the development of PK. Secondly due to lack of information on the active principle, it is difficult to select any particular compound or establish pharmacological basis for efficacy.

Further, the influence of dosage form, formulation aspect and/or type of extract of herbal medicines is also prominent on pharmacokinetics. For example, a recent open, single dose, crossover study was carried out to compare the pharmacokinetics of two *Ginkgo biloba* products from US market. Results showed that there was significant difference in all pharmacokinetic parameters between the test and reference products upon administration of equivalent doses. The reference product lead to higher plasma concentrations despite having less ginkgolides and bilobalide concentrations than test product (Kressmann 2001). Therefore, pharmaceutical equivalent products may not always be bioequivalent in case of herbal medicines. The multiple compounds present in different formulations lead to different kinds of metabolic and pharmacokinetic interactions leading to variable PK profiles.

While analyzing biological samples containing herbal medicines, one must keep in mind the extensive metabolic biotransformation that compounds may undergo. The evaluation of integral metabolism profile of herbal medicines has been an important breakthrough in predicting and explaining their pharmacokinetics, efficacy, herb-drug interactions as well as toxicity (Raskin et al. 2002; Li et al. 2009; Tang et al. 2009). The metabolism profile can reveal changes occurring in vivo due to herbal exposure. However, integral metabolism of herbal medicines is still largely unexplored, requiring exhaustive research.

Another challenge which is beyond the scope of traditional research is selection of analytical tools for PK elucidation of integral metabolism profile of complex herbal medicine present in a complex biological sample (e.g., blood, tissues, or urine). In a herbal medicine containing, for example, up to 300 compounds, the concentration of a particular compound in the single dose of a finished product can be in the lower mg range and the consequent plasma concentrations may go as low as μg to pg per liter range. This makes not only quantitative but global qualitative analysis difficult. The evaluation of integral metabolism profile requires advances bioanalytical strategy and tools having sufficient sensitivity as well as selectivity. The absence of reliable analytical tools remained a challenge for a long time. Currently available chromatography and mass spectrometry (MS) based technologies like liquid chromatography-MS (LC–MS), gas chromatography-MS (GC–MS), and capillary electrophoresis-MS (CE–MS) have become important facilitators of PK studies of herbal medicine. LC–MS or LC–MS/MS based assays have been commonly employed for analyzing multiple constituents of herbal medicines (Wang et al. 2008; Xin et al. 2011).

However, despite the technological advancement in the study of metabolites of single component herbal medicines, fate of multicomponent herbal medicines still remains a challenge. The obstacles in the development of metabolic profile of multicomponent herbal medicines are many. Firstly, it is difficult to standardize the chemical composition of multicomponent herbal medicines or to neutralize the effect of batch to batch variation. Secondly, in some instances there is an overlap between the chemical composition of normal diet and herbal medicines which can lead to pseudo results. Thirdly, extensive microbial-mammalian cometabolism of multiple components can occur in the gut which varies from species to species. Fourthly, multicomponent products further complicate the analytical differentiation between exogenous metabolites from the endogenous ones. Lastly, there are many overlapping metabolic pathways for chemically similar components. Sometimes, such pathways may intercross each other as well.

Methods for Pharmacokinetic Evaluation

A few methods have been proposed for the pharmacokinetic evaluation of multicomponent herbal medicines. The classical strategy has been to study the pharmacokinetics of single constituent of a multicomponent agent on individual biological reactions, genes, enzymes, etc. followed by assembling the findings to develop the comprehensive profile. However, the PK of any constituent in isolation may differ significantly from its behavior in a multicomponent system due to drug-drug interactions. It is practically not possible to

identify each metabolite in the global pool of metabolites and assess their effect on biochemical pathways. Further, implementation of this reductionist approach is not valid and does not capture the complex behavior from systems biology perspective (Xue and Roy 2003).

Hao et al. proposed the method for integrated PK study of multicomponent herbal medicines (originally proposed for traditional Chinese medicine) called the "AUC weighting integrated method" (Hao et al. 2009). The method includes following steps:

- Assessment of PK properties and therapeutic effects of each ingredient in multicomponent herbal medicine
- Selection of powerful ingredients with suitable PK characteristics as the PK markers
- Determination of blood drug concentration of the selected PK markers and plotting the blood drug concentration–time curves of the multiple components
- Integration of blood drug concentration–time profiles of each PK marker to represent the whole in vivo process of the multicomponent herbal medicine based on possible contribution weight of each marker to the pharmacological effect

Pharmacokinetic study of a herbal medicine preparation (Xuesaitong injection) is reported based on this scheme. Elucidation of identified five main PK markers viz. notoginsenoside R1, and ginsenosides Rg1, Re, Rb1, and Rd, and therapeutic effect of the same was carried out. The blood drug concentration–time profiles of these markers were subsequently investigated. The ratio of $AUC_{(0-\infty)}$ of each ingredient and sum of the $AUC_{(0-\infty)}$ of five markers was considered as the PK weight coefficient of each ingredient. Finally, sum of the value of the blood drug concentration X $AUC_{(0-\infty)}$ weight coefficient of each marker was regarded as the integrated blood drug concentration of the five markers at each sampling time point. This value was used to draw integrated drug concentration–time curve and estimate the integrated PK parameters as an indicator of whole PK properties of the preparation (Li et al. 2008).

Integrated PK profile of *Saussurea laniceps* have been reported using similar method (Yi et al. 2014). Although this method has been reported in more than one type of studies, there is argument that PK markers selected using this method might not comprehensively indicate therapeutic response of multicomponent herbal medicine (Liu et al. 2009). This is so mainly due to the fact that contribution "weight" of few of the multiple constituents to the overall therapeutic property is less known.

An ideal technique should not only indicate the integrated blood drug concentration profile but should also indicate the dose-dependent therapeutic effect of multicomponent herbal medicine. Such a method can help in carrying out simultaneous PK and PD evaluation. This can also help in utilization of PK profile in design of dosage regimen for herbal medicines. The currently available advanced technologies like liquid/gas chromatography coupled with mass spectrometry (LC-MS and GC-MS) as well as capillary electrophoresis coupled with mass spectrometry (CE-MS) and nuclear magnetic resonance (NMR) have made simultaneous detection of various metabolites of herbal medicines possible (Tolonen et al. 2009) through study of complete set of metabolome (Metabolomics). Metabolomics is a unique platform for poly PK studies which not only identifies and measures multiple constituents of herbal medicine *in vivo* but also characterizes any metabolic alterations upon human exposure (Sumner et al. 2007; Wang et al. 2005). Metabolomics has been recently utilized for the investigation of herbal medicine efficacy and toxicity (Xie et al. 2008; Ni et al. 2008; Chen et al. 2007). A proof-of-concept PK study of Pu-erh tea intervention has been reported based on metabolomics approach using tandem mass spectrometry (MS/MS) (Lan and Jia 2010). Metabolomics combined with statistical tools can address the challenges encountered in PK of multicomponent herbal medicines and in the assessment of their efficacy. Complete panel of dynamic pharmacokinetic profile for dosage regimens of multicomponent

herbal medicines may also help in minimizing overdosing and consequently reduction of toxicity.

Acknowledgements The views expressed by the authors in this book chapter are personal and not necessarily convey the view point of Central Research Institute of Unani Medicine or CCRUM.

References

Anonymous (2012) Global herbal supplement market to reach $107 billion by 2017. Nutraceuticals World

Anonymous (2017) New trends & effective strategies for medicinal & natural products. 5th international conference and exhibition on pharmacognosy, phytochemistry & natural products. http://pharmacognosy-phytochemistry-natural-products.pharmaceuticalconferences.com/events-list/herbal-drugs-and-formulations

Barnes PM, Bloom B, Nahin RL (2008) Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Rep 12:1–23

Bothwell LE, Podolsky SH (2016) The emergence of the randomized, controlled trial. N Engl J Med 375 (6):501–504. https://doi.org/10.1056/NEJMp1604635

Chan K (1995) Progress in traditional Chinese medicine. Trends Pharmacol Sci 16(6):182–187

Chan MF, Mok E, Wong YS, Tong TF, Day MC, Tang CK, Wong DH (2003) Attitudes of Hong Kong Chinese to traditional Chinese medicine and western medicine: survey and cluster analysis. Complement Ther Med 11(2):103–109

Chen X, Ye Y, Cheng H, Yin J, Sun C (2007) Comparative analysis of theanine, caffein and polyphenolic constituents in green tea, black tea and puer tea. Food Res Dev 12:046

EMEA (2007a) Procedure for the preparation of community monographs for herbal medicinal products with well-established medicinal use. European Medicine Agency, London

EMEA (2007b) Procedure for the preparation of community monographs for herbal medicinal products with well-established medicinal use. Committee on Herbal Medicinal Products (HMPC, London)

Ernst E, Schmidt K, Wider B (2005) CAM research in Britain: the last 10 years. Complement Ther Clin Pract 11(1):17–20

Gobburu JS, Gopalakrishnan M (2006) Optimal dose finding in drug development. In: Dose optimization in drug development, Drugs and the pharmaceutical sciences. Informa Healthcare, pp 195–215. https://doi.org/10.1201/9781420018585.ch9

Hao HP, Zheng CN, Wang GJ (2009) Thoughts and experimental exploration on pharmacokinetic study of herbal medicines with multiple-components and targets. Yao xue xue bao 44(3):270–275

Hollander JM, Mechanick JI (2008) Complementary and alternative medicine and the management of the metabolic syndrome. J Am Diet Assoc 108(3):495–509. https://doi.org/10.1016/j.jada.2007.12.007

Joos S, Glassen K, Musselmann B (2012) Herbal medicine in primary healthcare in Germany: the Patient's perspective. Evid Based Complement Alternat Med 2012:294638. https://doi.org/10.1155/2012/294638

Kabisch M, Ruckes C, Seibert-Grafe M, Blettner M (2011) Randomized controlled trials: part 17 of a series on evaluation of scientific publications. Dtsch Arztebl Int 108(39):663–668. https://doi.org/10.3238/arztebl.2011.0663

Koonrungsesomboon N, Karbwang J (2016) Ethical considerations in clinical research on herbal medicine for prevention of cardiovascular disease in the ageing. Phytomedicine 23(11):1090–1094. https://doi.org/10.1016/j.phymed.2015.10.017

Kressmann S (2001) Comparison of herbal medicinal products from Ginkgo biloba from the U.S. market. University of Frankfurt, Frankfurt

Lan K, Jia W (2010) An integrated metabolomics and pharmacokinetics strategy for multi-component drugs evaluation. Curr Drug Metab 11(1):105–114

Li X, Hao H, Wang G, Sun J, Liang Y, Xie L, Zhang Y, Yan B (2008) Integrated pharmacokinetic study of multiple effective components contained in total Panax notoginsenosides. Chin J Nat Med 6(5):377–381

Li Y, Duan J, Guo T, Xie W, Yan S, Li B, Zhou Y, Chen Y (2009) In vivo pharmacokinetics comparisons of icariin, emodin and psoralen from gan-kang granules and extracts of herba Epimedii, Nepal dock root, Ficus hirta yahl. J Ethnopharmacol 124(3):522–529. https://doi.org/10.1016/j.jep.2009.05.008

Liang YZ, Xie P, Chan K (2004) Quality control of herbal medicines. J Chromatogr Analyt Technol Biomed Life Sci 812(1–2):53–70. https://doi.org/10.1016/j.jchromb.2004.08.041

Liu H, Yang J, Du F, Gao X, Ma X, Huang Y, Xu F, Niu W, Wang F, Mao Y, Sun Y, Lu T, Liu C, Zhang B, Li C (2009) Absorption and disposition of ginsenosides after oral administration of Panax notoginseng extract to rats. Drug Metab Dispos 37(12):2290–2298. https://doi.org/10.1124/dmd.109.029819

Mukherjee PK, Bahadur S, Chaudhary SK, Kar A, Mukherjee K (2015) Chapter 1 – Quality related safety issue-evidence-based validation of herbal medicine farm to pharma. In: Evidence-based validation of herbal medicine. Elsevier, Boston, pp 1–28

Na DH (2010) Metabolism study of botanical drugs. Arch Pharm Res 33(12):1877–1879. https://doi.org/10.1007/s12272-010-1200-7

Ni Y, Su M, Lin J, Wang X, Qiu Y, Zhao A, Chen T, Jia W (2008) Metabolic profiling reveals disorder of amino acid metabolism in four brain regions from a rat model of chronic unpredictable mild stress. FEBS Lett 582

(17):2627–2636. https://doi.org/10.1016/j.febslet.2008.06.040
Ong ES (2004) Extraction methods and chemical standardization of botanicals and herbal preparations. J Chromatog Analyt Technol Biomed Life Sci 812(1–2):23–33. https://doi.org/10.1016/j.jchromb.2004.07.041
Parveen A, Parveen B, Parveen R, Ahmad S (2015) Challenges and guidelines for clinical trial of herbal drugs. J Pharm Bioallied Sci 7(4):329–333. https://doi.org/10.4103/0975-7406.168035
Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N, O'Neal JM, Cornwell T, Pastor I, Fridlender B (2002) Plants and human health in the twenty-first century. Trends Biotechnol 20(12):522–531
Routledge PA, O'Mahony MS, Woodhouse KW (2004) Adverse drug reactions in elderly patients. Br J Clin Pharmacol 57(2):121–126
Sumner LW, Urbanczyk-Wochniak E, Broeckling CD (2007) Metabolomics data analysis, visualization, and integration. Methods Mol Biol 406:409–436
Suresh K (2011) An overview of randomization techniques: an unbiased assessment of outcome in clinical research. J Hum Reprod Sci 4(1):8–11. https://doi.org/10.4103/0974-1208.82352
Tang J, Song X, Zhu M, Zhang J (2009) Study on the pharmacokinetics drug-drug interaction potential of Glycyrrhiza uralensis, a traditional Chinese medicine, with lidocaine in rats. Phytother Res 23(5):603–607. https://doi.org/10.1002/ptr.2450
Tilburt JC, Kaptchuk TJ (2008) Herbal medicine research and global health: an ethical analysis. Bull World Health Organ 86(8):594–599
Tolonen A, Turpeinen M, Pelkonen O (2009) Liquid chromatography-mass spectrometry in in vitro drug metabolite screening. Drug Discov Today 14(3–4):120–133. https://doi.org/10.1016/j.drudis.2008.11.002
USFDA (2016) Botanical drug development – guidance for industry. USFDA, Silver Spring
USFDA (2017) Multiple endpoints in clinical trials guidance for industry. USFDA, Sliver Spring
Vickers A, Zollman C (1999) ABC of complementary medicine: herbal medicine. BMJ 319(7216):1050–1053
Wang BL, JP H, Tan W, Sheng L, Chen H, Li Y (2008) Simultaneous quantification of four active schisandra lignans from a traditional Chinese medicine Schisandra chinensis (Wuweizi) in rat plasma using liquid chromatography/mass spectrometry. J Chromatog Analyt Technol Biomed Life Sci 865(1–2):114–120. https://doi.org/10.1016/j.jchromb.2008.02.016
Wang Y, Tang H, Nicholson JK, Hylands PJ, Sampson J, Holmes E (2005) A metabonomic strategy for the detection of the metabolic effects of chamomile (Matricaria recutita L.) ingestion. J Agric Food Chem 53(2):191–196. https://doi.org/10.1021/jf0403282
WHO (2005) National Policy on traditional medicine and regulation of herbal medicines. World Health Organisation, Geneva
WHO (2008) Essential Medicines – Biennial Report. World Health Organisation, Geneva
Wu JT, Zeng H, Qian M, Brogdon BL, Unger SE (2000) Direct plasma sample injection in multiple-component LC-MS-MS assays for high-throughput pharmacokinetic screening. Anal Chem 72(1):61–67
Xie G, Plumb R, Su M, Xu Z, Zhao A, Qiu M, Long X, Liu Z, Jia W (2008) Ultra-performance LC/TOF MS analysis of medicinal Panax herbs for metabolomic research. J Sep Sci 31(6–7):1015–1026. https://doi.org/10.1002/jssc.200700650
Xin GZ, Qi LW, Shi ZQ, Li P, Hao HP, Wang GJ, Shang J (2011) Strategies for integral metabolism profile of multiple compounds in herbal medicines: pharmacokinetics, metabolites characterization and metabolic interactions. Curr Drug Metab 12(9):809–817
Xue T, Roy R (2003) Studying traditional Chinese medicine. Science 300(5620):740–741. https://doi.org/10.1126/science.300.5620.740
Xutian S, Zhang J, Louise W (2009) New exploration and understanding of traditional Chinese medicine. Am J Chin Med 37(3):411–426. https://doi.org/10.1142/s0192415x09006941
Yi T, Zhu L, Tang YN, Zhang JY, Liang ZT, Xu J, Zhao ZZ, ZL Y, Bian ZX, Yang ZJ, Chen HB (2014) An integrated strategy based on UPLC-DAD-QTOF-MS for metabolism and pharmacokinetic studies of herbal medicines: Tibetan "Snow Lotus" herb (Saussurea laniceps), a case study. J Ethnopharmacol 153(3):701–713. https://doi.org/10.1016/j.jep.2014.03.031
Zamiska N (2006) On the Trail of Ancient Cures. https://www.wsj.com/articles/SB116354914377323235