PHCOG MAG.: Recent advances in Assay methods and techniques Preclinical safety studies on medicinal plants: Part 1

Arun Kumar H.S.
Rudolf-Buchheim-Institute for Pharmacology,
Frankfurter Strasse. 107, D-35392 Giessen,
Germany.
E mail: arun.kumar@phcog.net

Abstract
The use of various herbal remedies and preparations are described throughout human history representing the origin of modern medicine. Many conventional drugs originate from plant sources and some of the most effective drugs known today are plant based. As the interest in phyto-medicines and the use of plant derived extracts increases there is a need to improve not only quality control methods but also authentication methods in terms of safety and efficacy. An understanding of approaches to safety with regard to medicinal plant use can serve in the implementation of programs combining scientific and traditional knowledge and practice to enhance the safety and efficacy of medicinal plant use. In this regard studies to examine the side effects considering the interaction of herbs and modern drugs and assessing the risk-benefit ratio of the herbal drugs is need of the hour. The advantages of traditional systems of medicine with respect to their safety and efficacy could result in rational utilization of our rich herbal resources with application of the scientific methods.

Key Words: Medicinal plants, drug discovery, Safety, Toxicity testing.

Introduction

Herbal medicine, also referred to as botanical medicine or phytomedicine, is defined as the use of whole plant or part of plants to prevent or treat illness (1, 2). Scientific interest in medicinal plants has burgeoned in recent times due to increased efficiency of new plant-derived drugs, growing interest in natural products and rising concerns about the side effects of conventional medicine(3, 4). Based on current research and financial investments into medical plants, it seems that they will continue to play important roles in human health. Herbs are frequently used to treat or prevent a range of health problems. The use of herbal medicines has become a part of the mainstream all over the world while, some use herbal products with conventional medications, others use them as a replacement. Since herbal medicine addresses an important health-care need, its continued integration into mainstream medicine should benefit everyone (1, 5). It is estimated that 80% of the worldwide population use herbal medicines (3, 4), mainly as self-prescribed products and health food shops for herbal remedies are now considered equivalent to conventional drugstores (3, 6). The predominant attitudes toward the utility of medicinal plants often fall into two categories: uncritical enthusiasm or uninformed scepticism. However, a rational, analytical perspective is need of the hour to address the value and safety of these medications and the potential incompatibilities associated with co-use of medicinal plants and modern pharmaceuticals. Hence, education and training in scientifically validated screening, pharmacovigilance as well as an improved regulatory system for herbal medicine is of current priority.

Natural product drug discovery

While the discovery of medicinal drugs from natural products isn't new, the newer approaches to modern drug discovery from natural sources with the emphasis and the novelty in the biological test systems are in vogue. Wherein greatly expanded activity and resources are being focused on selecting specimens for screening. Some of the best drugs we have today are from natural products (1, 4). Over billions of dollars worth of natural products are consumed world wide annually (2, 5). The development of drugs from plants involves large-scale pharmacological screening of herbs. Herbal or natural products are becoming popular which can be attributed to the belief that they are safe. The herbal drug industry is considered to be a high growth industry and seeing the growing demand, it is all set to flourish in the next century (5). The trend for the increasing popularity of medicinal herbs globally demands its scientific validation as the toxicities and potential drug interactions of herbal medicines are often not or insufficiently validated.

Purity and standardization of natural product extract is always an issue due to the high variability, largely due to the variability in the source (4). Dosing of herbal preparations also varies and is dependent on factors such as growing and harvesting conditions, plant parts, and extraction methods. Hence standardization for active constituent in the herbs is important. The concept of standardized extracts definitely provides a solid platform for scientific validation of herbals as standardized extracts provide a consistent and effective alternative to crude drugs, as they constitute calculated ratio of the active constituents (7). The concept of standardization is now being widely welcomed and it is a rational way to increase the acceptance of medicinal herbs among the physicians and patients. These standards are of further importance as they control the nature of the extract to be manufactured.

Poly-herbal formulations as mentioned in classical texts of Ayurveda, are used by number of pharmaceutical companies (8, 9). It is very difficult to understand the theme of poly-herbal preparations as a number of ingredients may vary from two to 25 or

more. It is very difficult to set the standards of these types of formulations. The situation is further complicated by the multiple uses of these formulations as mentioned in Ayurveda. Keeping in view the growing popularity of herbals, it is the need of hour to have analytical data, bio-equivalence, pharmacological and toxicological studies with particular reference to herbal-synthetic drug interactions.

The major hindrance in the amalgamation of herbal medicines into modern medical practices is the lack of scientific and clinical data, and better understanding of efficacy and safety of the herbal products (7, 10). Ensuring the quality and safety of the herbal medicines is a priority and systematic approach through experimental and clinical validation of efficacy which is envisaged for traditional medicines (1, 4). As done in modern medicine, efficacy testing of the herbal products in experimental screening method and animal toxicity studies is important to establish the active component from appropriate extract of the plant and understand the potential adverse effects (7). Apart from the plant constituents, contaminants from the source materials such as microbes, microbial toxins, environmental pollutants, or heavy metals should also be checked in herbal medicine, as most often they could be responsible for the adverse effects. Unfortunately and astonishingly evaluation of toxicity/safety and adverse drug reaction of the herbal preparation is a neglected area, as herbs are considered natural products and, therefore safe. This lack of information makes it difficult to compare the benefit-risk profile of herbal medicines. The safety issues of herbal medicines are apparent in following aspects: (1) administration of an overdose; (2) administration over too long a time period; (3) unsuitable combination of one drug with other drugs; (4) lack of adequate authentication of different botanical drugs used, resulting in the use of adulterated medicines; (5) Inadequate preparation of the traditional remedies and (6) patients individual differences in drug reaction, pharmacogenetics, metabolism and allergic reactions (7, 10). Several supplements can interact with prescription and over-the-counter drugs. Such interactions may intensify or reduce the effectiveness of a drug or cause a serious side effect (11, 12). However documentation and scientific research on such adverse interactions is greatly lacking. Hence, well-designed studies to investigate supplement-drug interactions are need of the hour.

Safety Studies

During the past 50 years the emphasis on toxicology, has shifted from studying the mere toxicity of chemicals to the evaluation of its safety and natural products are no exception to it. All Medicines or pharmaceuticals irrespective of their source must be evaluated for its efficacy and safety before its intended use in humans or animals. Pharmacologic and toxicological data on such new chemical entities (NCE) from animal (preclinical) studies are submitted to the regulatory bodies as part of an application for an investigational new drug (IND). The pharmacokinetic, pharmacodynamic, and toxicologic properties of a drug must be evaluated and documented in animals, according to the recommendations from regulatory agencies under strict Good Laboratory Practices. In all such studies two main assumptions are made: The effects of chemicals in laboratory animals apply to humans; and the use of high doses in these animals is a necessary and validated method for discovering possible toxicity in humans. High doses are necessary because of the relatively small number of animals used and the need to detect low-incidence toxic responses. The safety of a drug is determined by studying the acute, subchronic, and chronic toxicities of the drug in several animal species and it must comply the regulatory requirements. A brief description of these studies is mentioned below:

Acute Toxicity Testing
These tests assess the potential of a toxicant to cause adverse effects after a single dose or multiple doses during a 24-hour or shorter period. At least two species (rodent and non rodent) are to be used with two routes of administration (one by intended route of use).

Subchronic Toxicity Testing
Groups of male and female animals are treated with the substance via oral or parental route at different dose levels daily for 14/28/90 days. Satellite groups are included to check the toxicokinetic parameters, reversibility and persistence of effect.
Study measurements include daily clinical observations, weekly body weights and feed consumption, ophthalmic examination, haematology, serum clinical chemistry, and urinalysis at pretest, interim and before termination.
Complete necropsy is performed on any animal that dies or upon termination, and weights are recorded for major organs. Full histopathological examinations are conducted.

Chronic Toxicity Testing
The test assesses the potential of the substance to cause possible adverse effects after prolonged exposure:
1.5 years in mice.
2 years in rats.
3 or more years in dogs.
Included are tests for **carcinogenicity**.
Groups of male and female animals are treated with the test substance for 80/104 weeks. Satellite groups are also included for evaluation of non-neoplastic toxicity for 52 weeks.
Study measurements include: weekly clinical observations and body weights recording for 13 weeks and monthly thereafter; weekly food and water consumption and ophthalmological examination, pretest and before study termination.
Hematology, clinical chemistry, gross and histopathological examinations are conducted to assess the organ specific toxicity and analysis of neoplastic and non-neoplastic lesion.

Reproductive Toxicity Testing
Here the drug/NCE is evaluated for its effects on:
Ovulation, conception, implantation, embryo and fetal development, length of gestation, parturition, lactation and early postnatal growth. *Usually 2 or 3 successive generation (rat) studies are done*.
Males and females are exposed to the drug/NCE starting from 60 days before breeding until the end of gestation and lactation.
The offspring are dosed from weaning through their complete reproductive cycle, including lactation.

Teratogenicity Testing

Teratogen: An agent which, when administered during gestation, produce *nonlethal structural or functional changes* in the embryo or fetus.

Teratogenicity testing is usually separated from chronic reproductive toxicity tests.
The aim is to administer the drug/NCE during the period of differentiation of fetal organs and tissues: e.g., mice exposed during days 6 to 15 of gestation; rats during days 6 to 18.

Mutagenicity Testing
Mutagenesis: Mutagenesis refers to induction of changes in DNA. This includes observable structural changes in the chromosomes, although changes in DNA are more often evident only at the biochemical level with no obvious structural alterations in the chromosomes.
The different types of mutagenicity tests followed are:
Dominant lethal test: Male animals are treated with the test compound and females are examined for an increase in pre- and post-implantation losses.
Cytological test: Animals are exposed to test compound and cells (susceptible cells i.e., rapidly dividing cells of bone marrow, germ cells, etc.) are examined for mutations (chromosomal aberrations).
Ames Test: This test involves use of mutant *Salmonella* bacterium that requires histidine for growth. If "back mutation" occurs, the organism grows in histidine-free media. Test compounds are often metabolically activated to a reactive form through exposure to liver cells, liver extracts, or hepatic enzymes, to assess if the metabolites have any mutagenic effects.
Host-mediated assay: Animal is given both the test agent and the mutant *Salmonella*. Host may metabolically activate the compounds leading to "back-mutation" in the *Salmonella* thus facilitating its propagation in histidine free media.

Irritation tests: The formulated drug/NCE products are evaluated by animal studies wherein they are tested with animal irritation tests. Studies include Draize eye irritation test, Draize skin irritation tests, and other irritation tests depending on the intended use.

Concluding remarks
Traditional systems of medicine have been used throughout the world for centuries. Certain ancient systems, such as Ayurveda, traditional Chinese medicine and Tibetan medicine, are still used extensively, particularly in their country of origin. In the west, interest in the therapies of such systems, particularly for the treatment of chronic illness, is growing. Unfortunately most of them have not been studied scientifically, and nearly all are unregulated. Because such dietary supplements are natural, many assume that they are safe to use. An understanding of approaches to safety with regard to medicinal plant use can serve in the implementation of programs combining scientific and traditional knowledge and practice to enhance the safety and efficacy of medicinal plant use in traditional medicine practice. Studies should be conducted to examine the side effects considering the interaction of herbs and modern drugs and assessing the risk-benefit ratio of the herbal drugs is need of the hour. The advantages of traditional systems of medicine with respect to their safety and efficacy could result in rational utilization of our rich herbal resources with application of the scientific methods.

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