CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

India is one of the 12 mega biodiversity zone covering 2.4% of world’s area but with 8% of global biodiversity. It includes 15 agro-climatic zones containing about 47,000 plant species including nearly 15,000 medicinal plants [1]. Several traditional healthcare systems exist in India from centuries and out of all the traditional practices, Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy are the official traditional systems of medicine. These systems are collectively known as Indian Systems of Medicine (ISM) [2].

Ayurveda is one of the most ancient system of traditional medicine of the world, has been practiced in Indian subcontinent since 5000 BC [3, 4]. It is a holistic approach towards life, health and disease management through medicinal herbs, minerals, diet, lifestyle and spirituality. Ayurveda was developed through daily experiences and mutual relationship between people and nature and thus not only cure diseases but also prevent disease, maintain health and promote longevity [5]. This holistic system looks at the whole person as a combination of body, mind and soul [6]. Therefore, it is a comprehensive and integral medicinal system, gift of Indian sages to mankind [7]. Ayurveda is widely respected for its uniqueness and global acceptance as it offers natural ways to treat diseases and promote healthcare [8]. Generally Ayurvedic formulations are multi-component mixtures, containing plant and animal – derived products, minerals and metals. Ancient’s texts like ‘Rig Veda’, Atharva Veda, and Official compendia like Ayurvedic Pharmacopoeia, Ayurvedic Formulary show dominance of plant – derived products [9].

Variations in geographical landscaping and biodiversities in the Indian subcontinent have helped to develop the use of a variety of plant species and other natural resources for health care and contributed to the Materia Medica of traditional systems of medicine. More than 25,000 single or polyherbal formulations are used by the tribal and rural population in
Export – Imports bank reports reveals that, the global trade of plant derived and plant originated products of around US $62 billion (with a growth of 7% per annum) where India holds stake US $1 billion. [10]. There is an urgent need to cherish these in both the National and International perspectives for benefit of mankind. Apart from healthcare, medicinal plant trade is an important alternative income generating source for underprivileged communities. Thus there is an urgent need of the scientific base evaluation of an ayurvedic system of medicine for accepting International regulatory guideline to get drug approval status.

1.1.1 International market scenario of Indian medicinal plants

In India, it is estimated that, there are about 25,000 licensed pharmacy of ISM. Presently about 1000 single drugs and about 3000 compound formulations are registered. Herbal industry in India uses about 8000 medicinal plants. From about 8000 drug manufactures in India, there are however not more than 25 manufactures that can be classified as large scale manufactures which includes Ansar Drug Laboratories, Surat, Acis Laboratories, Kanpur, Amil Pharmaceutical, New Delhi, Alrasin Marketing, Mumbai, Allen Laboratories, Kolkatta, Bhartirasanagar, Kolkatta, Dabur India Ltd., Ghaziabad, Dattatraya Krishan Sandu Bros., Mumbai, Herbals Pvt. Ltd., Patna, Herbo-med (P) Ltd., Kolkatta, The Himalaya Drug Co., Bangalore, Indian Herb and Research Supply co., Saharanpur, J and J Dechane Laboratories Pvt. Ltd., Hyderabad, Madona Pharmaceutical Reaearch Pvt. Ltd., Kolkatta, Kruzer Herbals, New Delhi, Shilpachem, Indore, Hamdard (Wakf) Laboratories, Delhi, Zandu Pharmaceutical Works Ltd., Bombay, Baidyanath Ayurveda Bhavan, Jhansi Charak Pharmaceuticals, Bombay [11].

Herbal industry shares about US $62 billion with good growth potential in the global pharmaceutical market. The World bank reports trade in medicinal plants, botanical drug products and raw material is growing at an annual growth rate between 5 and 15% [12]. India the value of botanical related trade is about US $10 billion per annum with annual export of US $1.1 billion [13] while China’s annual herb drug production is worth US $48 billion with export of US $3.6 billion [14]. India seems to be lagging behind and is ranked third in the herbal drug category, with less than 2% global market share. According to an Ayurveda expert, Chinese herbal medicines, which rarely contain 10% scientific base when
compared with the Indian Ayurvedic system, are doing better than India by 50-fold [15, 16]. However, India had failed to make an impact in the global market with drugs derived from plants and the gap between Indian and other countries is widening rapidly in the herbal field [17]. The export of herbal medicine from India is negligible despite the fact that, country has a rich traditional knowledge and heritage of herbal medicine. The constant challenges for the herbal product are lack of standardization, quality control and pharmacokinetic profile of bioactive molecules. Although the herbal medicines have been used for thousands of years, basic research programmers need to be focused on the quality assurance. To overcome contaminations from pesticide residues and heavy metals there should be a control measures to implement necessary SOP at source. Good laboratory practices (GLP), Good agricultural practices (GAP) and Good manufacturing practices (GMP) are needed to produce good quality medicinal products. Also scientific base with completed pharmacological data is required for the herbal system of medicine. Without all these measures, it is impossible to realize the dream of having a major share of herbal drug industry despite having gold mine of well documented and well-practiced knowledge of traditional systems of medicines. Traditional Chinese medicines (TCM) examples would help India at various levels including policies, quality standards, integration practices, research models and the complementary integration where public health is kept at the central position. Both India and China having great traditional systems of medicines with strong philosophical basis and could play an important role in new therapies, drug discovery and development process [18].

1.2 CHALLENGES TO AYURVEDIC SYSTEM OF MEDICINE

Although ayurvedic medicines, which mainly consists of polyherbal formulations are widely used for the prevention, diagnosis, treatment and management of diseases. Quality control and proper regulation worldwide are still a big challenge as shown in Fig 1.1 Widespread and growing use of botanicals has created a global challenge in terms of quality, safety and efficacy. Scientific validation and standardization of herbal medicines is needed for the future advancement of traditional medicine. Proper use of products of assured quality, could also do much to reduce any risks associated with herbal medicine. However, regulation and legislation of herbal medicines has been enacted in very few countries. Most of the countries do not have any proper regulation on botanicals and the quality of herbal products sold is generally not guaranteed [19, 20].
Establishing the pharmacological basis of efficacy of herbal products is a constant challenge due to their complex composition and the ever-increasing list of their putatively active constituents. *In vitro* assays normally are cheap and relatively easy to perform, but the relevance of the finding is based on a sufficient concentration of active constituents at the site of action. Of particular interest is the question of bioavailability to assess to what degree and how fast compounds are absorbed after oral administration of herbal products. Further elucidation of metabolic pathways, which yields potentially new active compounds and the assessment of elimination route and their kinetics. Thus the pharmacokinetic data become an important link data for pharmacological assays and clinical effects. There is usually detailed information available about the pharmacokinetics and biopharmaceutics of active pharmaceutical ingredients but it is absolutely lacking in the case of herbal medicine. The reason for that, lies in the complexity of extracts as multicomponent mixtures and lack of knowledge of the active principles. With increasing knowledge of putatively active compounds and availability of highly selective and sensitive analytical methods pharmacokinetics can be evaluated for the ayurvedic formulations. Thus this review promotes the urgent need of pharmacokinetics in the herbal system of medicine to achieve the uphold position in the global market.
1.3 PHARMACOKINETICS: A MISSING CRITICAL STEP IN THE HERBAL MEDICINE

Pharmacokinetics of the new drug entity is one of the regulatory requirements for an investigational new drug approval. However, for the majority of herbal formulations used in the traditional or conventional medical practice, data on their disposition and biological fate in human are lacking. It is vital in the drug development process to understand the absorption, distribution, metabolism and excretion (ADME) of an active molecule from these formulations and how they interact with conventional drugs before their launch in the market in order to ensure the rational use of herbal medicines.

Pharmacokinetics in the herbal medicines are largely ignored due to the rigor, cost and time consumption of the drug development process. One of the major reasons is that, for most of these multicomponent mixtures their active ingredients are not known. In addition, there is difficulties in measuring the quantities of the active constituents in systemic circulations due to very low concentrations, arising from the very small amount per dose in the final product. These challenges have led to the situation that, most herb-drug interaction studies and case reports in the literature only evaluate the outcome of adding a herbal product to an existing conventional drug therapy and monitoring changes in pharmacokinetics and clinical response on the traditional drug. [21] The metabolism of a drug, can be altered by another drug or foreign chemical; such interactions are significant in clinical effects.

Cytochrome P450 enzymes, a super family of enzymes found mainly in the liver are involved in numerous interactions between drugs, food, herbs and other chemicals. The observed induction and inhibition of the CYP enzymes by natural products in the presence of prescribed drug has led to the general acceptance that, natural therapies can have adverse effects contrary to the popular beliefs in countries, where there is an active practice of traditional medicine. Majority of the classes of conventional drugs have been shown to be affected by different types of herbal preparations leading to various consequences, including treatment failure, adverse / toxic effects and even death. In order to improve the safety of herbal system of medicine use alongside conventional therapies in public healthcare, it is necessary to know how the herbal drugs interact with conventional drug, early in the drug development. It is also necessary to predict early, so as to eliminate regulatory obstacles and
avoid market pressure for recalls that may have been induced by adverse effects linked to interactions. Therefore a better understanding of the pharmacokinetics of herbal formulations is needed to support the predictability of herb – drug interactions.

### 1.3.1 Bio-activity guided pharmacokinetic methods for herbal medicines

Conceptually, all active compounds in a pharmaceutical product that are bioavailable in the systemic circulation can be considered as relevant markers for quality control, since such markers import *in vivo* relevance. Unlike a conventional pharmaceutical product, which usually contains one absorbable active compound and is also a designated marker compound for QC, many compounds exist in polyherbal formulations. Thus identification of relevant marker (absorbable and bioactive) for herbal formulation becomes difficult, especially if they are determined by an *in-vivo* approach, eg. from blood or plasma samples after ayurvedic formulation administration. The difficulty is primarily technical as it is virtually impossible to distinguish many such markers from hundreds of endogenous chemicals which are also present in the systemic circulation. In addition, determination of specific activity of these marker compounds *in vivo* not only can be labor intensive, but require clinical facility as well as patient informed consent. Thus identification of relevant markers for polyherbal formulations is mandatory, for better clinical efficacy.

When polyherbal formulations are administered orally, which was the most common and convenient route of administration to achieve desirable therapeutic effect of the bioactive components must undergo the following biopharmaceutical/ pharmacokinetic processes in the gut; dissolution, metabolism and absorption [22]. Only those active components that are absorbable may be considered as relevant markers, since they are capable of contributing to *in vivo* activity. Thus, identification of absorbable bio-active components using *in vitro* models that can simulate *in vivo* PK process may be a practical approach for identifying relevant markers for quality control.

### 1.3.2 Non-compartment approach to pharmacokinetic analysis of polyherbal formulations

Theoretically, when the drug is rapidly distributed to all parts of the body, the body behaves as one compartment and the drug profile in the body can be described as one-compartment pharmacokinetic model. On the other hand, when the distribution of the drug
in a group of tissues or organs is faster than its distribution to other tissues or organs, the body behaves as two different compartments and the drug profile in the body can be described as two compartment pharmacokinetic model. The body can also behaves as multiple compartments, when the drug is distributed to different group of tissues, at different rates. The compartmental approach in data analysis, sometime is faced with difficulties such as, when the drug concentration-time profile after intravenous administration is described by a two compartment pharmacokinetic model, but after oral administration of the same drug, the profile is similar to that of the one compartmental model because the rate of absorption and distribution process are not distinctively different. To avoid these difficulties, a different approach, known as the non-compartmental approach, may be used for data analysis can be examined, need not to be assumed to certain compartmental model [23].

The non-compartmental approach in pharmacokinetic data analysis is based on the statistical moment theory, which is utilized in chemical engineering. This theory views the drug molecules in the body as randomly distributed and each molecule has certain probability to eliminate at certain time t. So, according to this theory, the time course for the drug concentration in plasma can be regarded as probability density function. This probability density function multiplied by time raised to certain power (0, 1 or 2) and integrated over time yields the area under the moment curve. For example, the area under the zero moment curve can be determined from equation 1.1, which is equal to AUC. Also, the area under the first moment curve (AUMC) can be determined according to equation 1.2. Only the areas under the zero and first moments are utilized in pharmacokinetics, because higher moments are subjected to large computational errors.

Area under zero-moment curve \[ \text{Area under zero-moment curve} = \int_{t=0}^{t=\infty} t^0 \, Cp \, dt = \int_{t=0}^{t=\infty} Cp \, t = \text{AUC} \]  
(Eq 1.1)

Area under first-moment curve \[ \text{Area under first-moment curve} = \int_{t=0}^{t=\infty} t^1 \, Cp \, dt = \int_{t=0}^{t=\infty} t \, Cp \, dt = \text{AUMC} \]  
(Eq 1.2)

The principle of moment analysis has been utilized to estimate pharmacokinetic parameters such as mean residence time (MRT), drug clearance and the volume of distribution at steady state.
1.3.3 Mean residence time

The drug molecules are distributed throughout the body after drug administration. Some drug molecules are eliminated from the body faster than other molecules, which stay longer despite the fact that all the molecules are similar. The difference in the residence time for each molecule in the body occurs by chance according to statistical moment theory. The MRT is defined as the average time for the residence of all the drug molecules in the body. MRT can be calculated from the area under zero and first moment curves according to equation 1.3.

\[
\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} \quad \text{(Eq 1.3)}
\]

Where AUMC is the area under the first moment – time curve and AUC is the area under the zero moment time curve or the area under the plasma concentration-time curve. The MRT has units of time.

The absolute bioavailability is determined from the ratio of AUC after oral and i.v administration as in equation 1.4 and the relative bioavailability of two products are determined from the ratio of AUC for the two different oral products as given in the equation 1.5.

\[
F_{\text{Absolute}} = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{iv}}} \quad \text{(Eq 1.4)}
\]

\[
F_{\text{Relative}} = \frac{\text{AUC Product A}}{\text{AUC Product B}} \quad \text{(Eq 1.5)}
\]

The drug clearance can be determined after i.v administration from the dose and the AUC as in equation 1.6 and after oral administration as in equation 1.7.

\[
\text{CL}_T = \frac{\text{Dose}_{\text{iv}}}{\text{AUC}_{\text{iv}}} \quad \text{(Eq 1.6)}
\]

\[
\frac{\text{CLT}}{F} = \frac{\text{Dose}_{\text{oral}}}{\text{AUC}_{\text{oral}}} \quad \text{(Eq 1.7)}
\]
Using non-compartmental approach for pharmacokinetic data analysis have successfully evaluated the pharmacokinetics of particular bio-active components for Visnagin and *Ammi visnaga* [24], Senkyunolide I [25] and many Chinese formulations.

Recognition of the medical and health benefits of ayurvedic system of medicine with health claim is growing worldwide. Pharmaceutical research must go beyond focusing on pharmacological efficacy of polyherbal formulations, but also in studies that improves their effectiveness, in order for humanity to fully benefit from their inherent therapeutic potentials. With advance in instrumentation like HPLC/MS/MS and HPLC/MS/NMR as increasing number of components are being identified [26] from starting material used for preparation of those products. India needs a clear policy for such integration without compromise on the strategies that are science-based. Efforts are needed to establish and validate pharmacoepidemiological evidence regarding safety and practice of Ayurvedic medicines.

Pharmacokinetic data of bio-active components contribute considerably to the scientific assessment of the various claims of herbal products, which are increasingly marketed with curative claims worldwide. While a registration process reviewing quality, safety and efficacy of herbal medicinal products is established in curtain European countries also the FDA is considering to review certain botanicals via the IND/NDA (Investigational New Drug/New Drug Application) process [27]. In summary Indian herbal system of medicine having great traditional with strong philosophical basis and could play an important role in new therapies, drug discovery and development process. Indian system of medicine with strong scientific evidence will be a top leader in the global market.

**1.4 PATENTED POLYHERBAL FORMULATIONS**

In the present study, we have taken two patented polyherbal formulations, The US patented polyherbal formulation consists of *Salacia oblonga, Salacia roxburghii, Garcinia indica* and *Lagestroemia parviflora* was developed for prevention and management of Type II Diabetes mellitus and its vascular complications associated with diabetes mellitus and another one is European patent polyherbal formulation for the management of cardiovascular and neurological disorders consists of leaves of *Bacopa monnieri* (Scrophuliariaceae), fruits of *Hipphophae rhamnoides* (Elaganaceae) and bulbs of *Dioscorea bulbifera* (Dioscoraceae) developed and obtained by Dubey et al [28] has been
investigated. The details of the polyherbal formulations were given in Table 1.1. The individual herbal extract of each formulation was prepared from the authenticated plant material. All the ingredients (Table 1.1) were collected, dried and powdered separately, passed through 45# sieve and then mixed together in specified proportions in geometrical manner to get uniform mixture. The formulation composition was formulated into capsule as per the patent information of the effective dose by M/s Varanasi Bio Research Pvt Ltd. Varanasi, India. The chemical structures of the active components are depicted in Figure 1.2-1.6

Table 1.1 Details of patented polyherbal formulations

<table>
<thead>
<tr>
<th>S.No</th>
<th>Polyherbal formulations</th>
<th>Formulation contains the extract of</th>
<th>Effective Dosage (mg per capsule)</th>
<th>Active components</th>
<th>Patent Details</th>
</tr>
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</table>
| 1    | Herbal formulation for prevention and management of Type II Diabetes mellitus and vascular complications associated with Diabetes mellitus | *Salacia oblonga*  
*Salacia roxburghii*  
*Garcinia indica*  
*Lagestroemia parviflora* | 162.5 112.5 87.5 112.5 | Mangiferin  
Ellagic acid  
Hydroxycitric acid | US Patented 8337911B2  
Dec 25, 2012 (20) D-Formulation |
| 2    | Herbal formulation for management of cardiovascular and neurological disorders           | *Bacopa monnieri*  
*Dioscorea bulbifera*  
*Hippophae rhamnoides* | 225 125 125 | Bacoside  
Diosgenin  
Quercetin and Rutin | EP1569666B1  
Dec 13, 2011 (21) N-Formulation |

Mangiferin (Xanthone glycoside), is one of the active component of *Salacia* species, was the major active constituent of Diabetic D - formulation for the management of diabetes [29]. Quercetin and Rutin flavonoids are the major constituents present in the Neuro N-formulation. Both these markers having related pharmacological actions like antioxidant activity such as modification of eicosanoid biosynthesis, prevention of low density lipoprotein from oxidation, prevention of platelet aggregation and promotion of relaxation of cardiovascular muscle [30]. Also it was proven that, Quercetin and its related bioflavonoids modulate the function and expression of P-glycoprotein and CYP3A4 which were primarily involved in the drug interactions [31].
Chemical structure of Bioactive compounds

Considering the emerging need of the standardization and pharmacokinetics for the Indian system of medicine particularly polyherbal formulations, the above patented polyherbal formulations were selected for the present study and it was planned to standardize, quality control of its product and particularly pharmacokinetics of the major bioactive components for the FDA drug status in global market was carried out.