1. INTRODUCTION

Herbs have been used to treat various ailments since medicine began. Nowadays, 80% of the world’s population uses medicines which are directly or indirectly derived from plants [1]. Rapid growth has been seen in herbal medicinal products (HMP’s) market in recent years, as increasing number of consumers are persuaded by the benefits of plant extracts as an alternative to medicinal products with chemically derived active pharmaceutical ingredients (APIs). HMP’s have to meet comparable standards concerning the assessment of efficacy, safety and bio pharmaceutical quality as chemically defined synthetic drugs. In contrast to chemically defined drug products the biopharmaceutical quality and behavior of HMP’s are not well documented. In most cases in vitro / in vivo biopharmaceutical characterization is complicated by complex composition of herbal drug preparations, extensive metabolism of their constituents and resulting analytical difficulties [2]. In regulatory process; the efficacy, safety and appropriate pharmaceutical quality are to be assisted for all medicinal products intended for human use. In case of solid oral dosage forms intended for systemic actions, the biopharmaceutical performance must also be characterized by suitable in vitro and in vivo investigations [3]. These global requirements are not only relevant for synthetic chemical entities, but also for herbal remedies. However, due to their complex composition, such information is not available at this moment for most of the herbal formulations. Despite of increased use of HMP’s by public and major health issues raised, the concern of plant scientists has been increased about these products to provide scientific evidence regarding quality, safety and efficacy of their multiple chemical constituents which are responsible for therapeutic action of HMP’s. The necessity of in vivo studies for a biopharmaceutical characterization of the product depends on the solubility and permeability properties of the active drug ingredients as well as the dissolution behavior of herbal formulation [4].

Unlike orthodox medicines, specific guidelines for dissolution testing of Complementary/alternate (CAMs) and traditional medicines (TM’s) have not been developed. Also, there is no dissolution testing requirements for the quality control of such products [5]. Evidence based verification of the efficacy of HMP’s is still frequently lacking. However, in recent years, data on evaluation of therapeutic activity and
toxicity of HMP’s became available. The advances in analytical technology have led to the discovery of many new active constituents. A better understanding of the pharmacokinetics can help in designing rationale dosage regimens [4].

In this regard, pharmacokinetic studies for HMP’s have been conducted and they should be critically evaluated by sensitive analytical methods to assess the elimination routes and kinetics. Knowledge of kinetics and dissolution techniques brings in vitro and in vivo correlation (IVIVC) comes into an account. IVIVC often used in pharmaceutical development in order to reduce development time and optimize formulations. A good correlation is a tool for predicting in vivo results based on in vitro data. IVIVC allows dosage form optimization with the fewest possible trails in man, fixes dissolution acceptance criteria and can be used as a surrogate for further bioequivalence studies. It is also recommended by regulatory authorities. So far, there is significant lack of experience and scientific knowledge regarding the biopharmaceutics of HMP’s. This situation is contrasted by substantial progress made by chemically defined drug products.

In the year 1995 the biopharmaceutical classification system (BCS) was introduced to categorize drugs based on their rate and extent of drug absorption is aqueous solubility and gastro intestinal permeability [6]. Over a decade, the World Health Organization (WHO), US Food and Drug Administration (US-FDA) and European Medicines Agency (EMA) have implemented BCS for setting standards for market approval of drugs with special reference to oral immediate release (IR) dosage forms. For in vivo testing of IR oral solid dosage forms, FDA granted BCS i.e., a biowaiver can currently be requested only for solid, orally administered immediate-release products (85% release in 30 min) [7, 8]. IR drugs have been provisionally classified according to BCS were included in WHO essential medicines list, 200 drugs from US, UK, Spain, Japan and 135 national essential medicines from Pakistan [9-11].

In order to classify the drugs according to BCS, the solubility and permeability of the drug should be known. The IR dosage forms should show > 85% release in 30 min, possess highest solubility over the pH range 1 to 7.5 (dose/solubility ratio < 250 ml), higher permeability (fraction absorbed > 90 %) and excipients should not affect the absorption rate. Drug products with a narrow therapeutic range and drugs absorbed in the oral cavity are not considered for biowaiver.
The BCS classifies drugs into 4 different classes.
Class I- Drugs with high solubility and high permeability
Class II- Drugs with low solubility and high permeability
Class III- Drugs with high solubility and low permeability
Class IV- Drugs with low solubility and low permeability

The FDA adapted BCS for regulatory purpose. The drugs fitted in BCS provide information regarding post approval changes of generic product approval with out in vivo studies. Based on BCS waivers for invivo testing of IR oral solid dosage forms, class I can be granted if in vitro dissolution testing for two products can be similar. As per WHO guidelines the term biowaiver refers to generic medicine based on dissolution criteria related to active pharmaceutical ingredient (API) as a surrogate for in vivo bioequivalence testing. Now more consideration was given to the biowaiver for class III drugs with rapid dissolution and low permeability and procedure has been included in EMEA Guidance [12]. For weakly acidic drugs WHO included a procedure for biowaiver if they dissolve rapidly at pH 6.8.

The HMP’s are unregulated in different regions of world and considered as dietary supplements in USA. Only Europe and Canada have regulations that require an approval. In China the registration of Chinese HMP’s is according to the drug administration law of the people’s republic of china. But in approval process traditional bioavailability/ bioequivalence have not been performed for traditional Chinese medicine when compared with western medicines. In case of HMP’s lack of consistency during manufacturing, content uniformity, different practices of usage of pesticides, heavy metal contamination, excipients inconsistency, harvesting time, location, other contaminants and incorrectly labelled herbal drugs are different in different countries which ultimately leads to adverse effects, toxicity and herb drug interactions. Hence, it is very essential to regulate pharmaceutical standards for HMP’s [13]. BCS for herbal markers have varied implications in many regions of the world. Due to less reference product establishment the phytoequivalence concept became theoretical to some extent. The fundamentals of BCS for HMP’s can be used to gain knowledge and helpful in setting in vitro quality standards about HMP’s.
1.1. Formulation and BCS Strategies of HMP’s

The BCS concept should be valid for herbal medicines with more than one ingredient and herbal products with more than one herb. The application of BCS to HMP’s is more complex compared to synthetic drugs with one or few API combinations with defined excipients matrix. Now a days, BCS is applicable to HMP’s in which the herbal marker compounds are classified based on BCS principles to establish dissolution standards, ensure consistency in orally used HMP’s with minimal cost and to set quality of in vitro standards there by achieve maximum therapeutic benefit worldwide. But the concept of BCS can be used to gain biopharmaceutical knowledge about herbal markers. BCS characterization of herbal markers can be useful in setting quality standards for HMP’s especially in designing disintegration tests for the herbal formulation with highly soluble constituents (class I and III) might only be required to meet specifications of disintegration but with poorly soluble constituents (class II and IV) need to pass a dissolution test for batch to batch consistency that demonstrates the approximate content. It is very difficult and expensive to obtain clinical safety and efficacy data and batch to batch consistency for HMP’s. When compared with synthetic drugs, the HMP’s quality are not well documented which is very essential. However, there is need at this time point for the assessment of biopharmaceutical quality of herbal drugs intended for oral use. Hence, a classification system for herbal drugs is developed based on herbal extract information by European Pharmacopoeia (EP) and International Pharmaceutical Federation (FIP).

Based on this classification system herbal extracts can be characterized into three categories.

**Class A:** Standardized extracts with constituents solely responsible for therapeutic activity (Milk Thistle, Senna)

**Class B:** Quantified extracts with constituents possessing active markers. (St. John’s Wort, Ginkgo)

**Class C:** Other extracts with no constituents documented as being relevant for efficacy or as having pharmacological or clinical relevance (Valerian).
Again these categories can be subdivided into *extracts with negative markers* substances that have to be limited due to their toxicity or *phyto equivalence markers* that might be used to establish bioequivalence between products (flavonoid glycosides of *Ginkgo biloba*). In Europe, the extracts of type A or B, BCS and biowaivers could be used to establish pharmaceutical equivalence for markers and post approval changes of HMP’s for *in vitro* stability demonstration, but not type C extracts because of no known active constituents [14]. The BCS concept may be helpful for upgradation of category C extracts into category B or A. The BCS takes into account three major factors that govern the rate and extent of drug absorption such as dissolution, solubility, and intestinal permeability.

**1.2. Practical Approach of BCS**

The important parameters needed for classification of drugs into BCS include dose number, solubility, permeability and dissolution. According to FDA biowaiver guidelines, absence of evidence suggests instability in gastrointestinal tract and a drug is considered highly permeable when the extent of absorption is 90% or more than administered dose in humans.

**Dose number (D₀)**

It describes the relationship between maximum dose strength and solubility.

\[
D_0 = \frac{M_0}{V_0} \times C_5
\]

Where \(M_0\) – Highest dose strength (mg); \(V_0\) – Volume of water taken with dose (250 ml); \(C_5\) – minimum physiologic solubility at pH 1.2, 4.5 and 6.8 at 37 °C (mg/ml).

In general, compounds with \(D_0\) value as less than 1 are considered as highly soluble.

**1.2.1. Solubility**

For an immediate release dosage form, solubility is defined as highest dose strength. A drug molecule is considered as highly soluble over the pH range of 1 to 7.5 when highest dose strength is soluble in aqueous media of 250 ml or less at 37°C. The protocol prescribed that administration of drug to human volunteers that are fasting with one glass of water (250 ml). The main objective of BCS is to determine the equilibrium solubility of drug under physiological pH 1 to 7.5. The pH conditions for the drug solubility are based on ionization characteristics of the drug used for the test. Minimum of three replicate solubility determinations in each pH condition is
recommended. For reliable estimate of solubility sometimes, additional replication may be required sometimes depending on study variability. The buffer solutions used for solubility studies should be prepared according to pharmacopoeia guidelines. By adding drug to the buffer solution pH should be verified. Methods like acid/base titration should be preferred with justification other than traditional shake flask method to predict the equilibrium solubility of test drug. A validated stability indicating assay is used to determine the drug concentration in selected buffers to distinguish drug from other degradation products. It should be reported along with stability data if there is any degradation of drug it is observed as a function of buffer composition or pH [15].

1.2.2. Permeability

The effective permeability is defined as units of molecular movement per unit time. High permeability drugs have extent of absorption greater than or equal to 90 % and not associated with gastrointestinal instability problems. The permeability class of a drug substance can be determined either in human subjects or in intestinal perfusion approaches by the methods described below [16].

- Pharmacokinetic studies in humans
  - Mass balance pharmacokinetic studies
  - Absolute bioavailability studies
- Intestinal permeability methods
  - *In vivo* intestinal perfusions studies in humans
  - *In vivo or in situ* intestinal perfusion studies in animals.
  - *In vitro* permeation experiments with excised human or animal intestinal tissue.
  - *In vitro* permeation experiments across epithelial cell monolayer’s

The primary source for permeability data is the fraction absorbed in human studies. In some cases the Caco-2 cell line results are taken along with human trials into account as additional evidence (cimetidine, ciprofloxacin, furosemide, phenoxymethylpenicill -in, phenytoin and propranolol). The animal data are considered in few exceptional cases (acetazolamide, benznidazole, furosemide and sulfadiazine). Data like oral
versus intravenous application, urinary recovery, radio labelled drugs and perfusion studies in humans are considered. The drugs whose bioavailability was compromised due to degradation in gastrointestinal track or due to first pass metabolism were marked with asterisk. In case of poorly soluble drugs it is difficult to determine the bioavailability < 90 % is due to problem in solubility or permeability. Some times when drug is administered with food the higher bioavailability is an indication for < 90 % of absorption was considered as solubility problem rather than permeability problem [17].

1.2.3. Dissolution methods

Dissolution method for marker compound

The 85 % of labelled claim of immediate release (IR) drug should dissolve within 30 min using United States Pharmacopoeia (USP) apparatus I (100 RPM) or apparatus II (50 RPM) in 900 ml volume or less in media like 0.1N HCl or Simulated Gastric Fluid (SGF) USP without enzymes at buffer pH 4.5 and 6.8 or Simulated Intestinal Fluid (SIF) USP without enzymes. Regulatory interest is to know the similarity between the two curves. To indicate the similarity FDA set the public standard f2 value between 50-100 [15]. For each profile determination minimum 12 units should be used. In case of mean dissolution data the earliest point % coefficient of variance should be less than 20% and other time points should be less than 10%. The pre and post dissolution measurements and dissolution time points of the two products should be performed under the same test conditions. For IR drugs 15, 30, 45 and 60 min, and for extended release (ER) products 1, 2, 3, 5 and 8 h are considered as time points of dissolution. Only one measurement should be considered after 85% of dissolution because the f2 values are sensitive to the number of dissolution time points. The profile comparision is not necessary for rapidly dissolving products i.e., more than 85% dissolution in 15 min or less. f2 value greater than 50 % indicates the equivalence of the two curves and further it denotes the performance of the drug products. Under circumstances wide variability is observed then for statistical evaluation, a boot strap approach is used to calculate confidence interval. According to FDA biowaiver the drugs are classified into BCS based on the above data.
**Dissolution method for herbal extracts**

In pharmaceutical development and quality control of the oral dosage forms of active pharmaceutical ingredients dissolution studies have been widely used [6]. Due to the occurrences of a great deal of potential active constituents, the drug dissolution is extremely difficult to be comprehensively investigated for herbal medicines [18]. The *in vitro* dissolution is an important measure to consider the bioavailable properties of the solid dosage forms of herbal medicines.

For type A HMP’s (class A) the *in vitro* release of the constituents with known therapeutic activity should be compared with the reference product. The *in vitro* release of the constituents with known therapeutic activity exceeds 90 % for both the products then pharmaceutical equivalence may be accepted. If the constituents were having low solubility or poor *in vitro* release then bioequivalence studies may be necessary.

For type B1 HMP’s (class B) the *in vitro* release of the active markers and the solubility of the extract should be compared with the reference product. If both parameters exceed 90 % for both the products then pharmaceutical equivalence may be accepted. In case of poor solubility and low *in vitro* release then additional clinical studies or bioequivalence studies are needed.

For type B2 HMP’s (class C) the solubility of the extract and should be compared with the reference product. If both parameters exceed 90% for both the products then pharmaceutical equivalence may be accepted. If the solubility of the extract cannot be tested then the appropriate marker release should be compared. If there is no appropriate marker then disintegration profiles may be accepted. Justification was required for not selecting an appropriate marker and why bioequivalence studies are not needed.

**1.3. BCS Applications in formulation development**

BCS is a simple tool useful in early development for determination of oral absorption in drug development process [6]. For IR drugs FDA grant a waiver for time consuming bioequivalence studies which reduced timelines in the drug development process. For class I drugs it is essential to achieve a target release profile with
pharmacokinetic/pharmacodynamic profile and formulation approaches like release rate control and properties like pH solubility profile of the drug are essential. In case of class II drugs the techniques needed are micronisation, lyophilisation, surfactant addition, micro emulsions systems and addition of complexing agents. The class III drugs require addressing the fundamental limitations of absolute permeability. Class IV drugs include major challenges in drug development and route of administration of such drugs include parental formulation with solubility enhancers. Consequently, it may be useful to extrapolate this experience to the concepts for development of formulations as shown in fig 1.

**Fig 1. BCS and concepts for viable formulation options**

1.4. Formulation strategies based on biopharmaceutics classification system

*Formulations for BCS class I drugs*

IR solid oral dosage forms, for example, conventional tablet or capsule formulations, are commonly designed to ensure rapid dissolution in the gastrointestinal tract [19].

*Formulations for BCS class II drugs*

Generally, the bioavailability of a BCS class II drug is rate-limited by its dissolution. Therefore, enhancement of dissolution rate of the drug is thought to be a key factor for improving the bioavailability of BCS class II drugs. Several physicochemical factors control the dissolution rate of drugs. Crystal modification [20], particle size
reduction [21], self-emulsification [22], pH modification [23], and amorphization [24] are considered to be effective for improving the dissolution behaviour of BCS class II drugs as shown in fig 2.

**Formulations for BCS class III drugs**
The bioavailability of BCS class III drugs is rate-limited by the membrane permeability in the gastrointestinal track. For BCS class III drugs, IR solid dosage forms should be practically designed for clinical use, although the absorption could be limited by membrane permeation. Permeation enhancers, such as fatty acid, bile salts, surfactants, and polysaccharides, play a role in enhancing the permeability of drugs via the paracellular pathway [25, 26].

**Formulations for BCS class IV drugs**
The formulation approaches similar to those for BCS class II and III approach could be practically applied to BCS class IV drugs, even though the absorption could be limited by the poor permeability after dissolving in the gastrointestinal track [27].

![Diagram of drug dissolution and formulation strategies](image)

**Fig 2. Different strategy for improvement of poor soluble drugs.**

**1.5. Delivery options for class IV drugs**
In drug discovery the combinatorial chemistry and high throughput screening often leads to new chemical entities with high molecular weight and increasing lipophilicity and therefore decreasing aqueous solubility [28, 29]. It is estimated that nearly 40% of the drugs in the pipeline have solubility problems and 60% of new drugs are poorly water-soluble [30]. To achieve its pharmacological activity, drug must be present in the dissolved state at the site of absorption in oral administration. Many
approaches have been developed to improve the drug solubility in aqueous phase, such as crystal modifications, salt formation, particle size reduction, amorphization, complexes with cyclodextrins, self emulsification, pH modification, nanocrystals and lipid formulations.

1.5.1. Crystal modifications

Metastable polymorphs
Polymorphism in crystalline solids is defined as materials with the same chemical composition, but different lattice structures and/or different molecular conformations. This is an effective approach for enhancing dissolution rate of the drug. These metastable forms eventually transform to thermodynamically stable form. However it is necessary essential to monitor polymorphic transformation during manufacturing and storage of dosage forms thereby ensuring reproducible bioavailability after oral administration.

Co crystal formation
Co crystal is broadly defined as crystalline materials comprised of at least two different components an API and a nontoxic guest molecule (co crystal former) in a stoichiometric ratio. pKₘₐₚ plays an important role in distinguishing salt to co crystal. When pKₘₐ value is less than 0 it can be defined as co crystal and when it is between 1 and 3 they can be distinguished as either salts or co crystals and even can contain sheared protons and mixed ionization states which can be assigned to either category.

Salt formation
In the pharmaceutical industry, salt formation approach is commonly used for an ionizable drug to increase solubility and dissolution rate. Salts are formed via proton transfer from an acid to a base. An appropriate salt form should be developed from the viewpoints of both physicochemical and biopharmaceutical properties, especially for poorly water-soluble drugs.

1.5.2. Particle size reduction

Micronization
Particle size reduction approach is widely used to increase dissolution rate as well as salt formation. Micronization approach successfully enhanced the bioavailability of
poorly water-soluble drugs. The common method to obtain micronized drug particles is mechanical pulverization of larger drug particles. Jet milling, ball milling, and pin milling are commonly used for dry milling.

**Amorphization**

Amorphous solids have higher energy than crystalline solids. Typically, the solubility of an amorphous drug is higher than that of the corresponding crystalline drug. Stable amorphous formulations can be obtained by solid dispersion techniques. Amorphous solid dispersion (ASD) is defined as a distribution of active ingredients in molecular and amorphous forms surrounded by inert carriers. ASD formulations can be prepared by spray drying, melt extrusion, lyophilization, and use of supercritical fluids with polymeric carriers and/or surfactant.

1.5.3. **Cyclodextrins complexation**

Cyclodextrins are oligosaccharides containing a relatively hydrophobic central cavity and hydrophilic outer surface. Cyclodextrins and their derivatives increase the apparent solubility of poorly water soluble drugs by forming inclusion complexes.

1.5.4. **Self emulsification**

Self emulsification formulations are isotropic mixtures of oil, surfactant, co solvent, and solubilized drug. These formulations can rapidly form oil in water (w/o) fine emulsions when dispersed in aqueous phase under mild agitation. They are additionally classified into self micro emulsification drug delivery systems (SMEDDS) and self-nanoemulsification drug delivery systems (SNEDDS) according to the size range of their oil droplets [31]. SMEDDS form microemulsions ranging in droplet size from 100 to 250 nm. Finer microemulsions of less than 100 nm can be obtained using SNEDDS.

1.5.5. **pH modification**

pH modification in solid dosage forms is considered to be an alternative option for an ionizable drug to improve the solubility and dissolution rate. These results suggested that pH modification in dosage form could reduce the variability in the absorption of administered drugs.
1.5.6. Nanocrystals

Reduction of particle size to nano meter range (< 1 μm) is a pretty good approach for poor water soluble drugs. Decreasing the particle size into nano meter is a promising approach to improve the apparent saturation solubility, dissolution rate and oral bioavailability of hydrophobic drugs. Compared to other nano-technological approaches, nanocrystals have a very high drug loading, as the particle core is composed of pure drug material. Decrease in particle size leads to decrease in diffusion layer thickness and increase in surface area and this leads to enhanced dissolution rate of a drug [32]. Even increase in saturation solubility leads to reduction in particle size (< 1 μm). The nanocrystal formulations are commonly produced by wet-milling with beads, high-pressure homogenization, or controlled precipitation. Hydrophilic polymer and/or surfactant are typically used to stabilize nanocrystal suspension. The nanocrystalline drug particles are dispersed into inert carriers after a drying process, such as spray drying or lyophilization. The solidified nanocrystal formulations can be defined as crystalline solid dispersion (CSD).

Among the various method described above for preparation on nano crystal, solvent/antisolvent precipitation technique are an effective technology in preparation of nano crystals. In this technique the drug is dissolved in a solvent, which is then added to non-solvent containing the surfactant as stabilizer under stirring at 3000 rpm. Precipitation of solid drug particles occurred immediately upon mixing. The suspension are centrifuged at 15,000 rpm for 20 min and washed twice with purified water. The precipitated nanoparticles are oven-dried at 35°C for 24 h. The basic advantage of precipitation technique is the use of simple and low cost equipments.

1.5.7. Lipid formulations

Solid lipid nanoparticles (SLN) are lipid based submicron particulate colloidal carrier systems ranging in size from 10-1000 nm [33]. SLNs combine advantages of other colloidal carrier systems like liposomes, polymeric nanoparticles and emulsions, but at the same time avoid or minimize the drawbacks associated with them [34]. ‘Lipid’ systems have the advantage that they can present the drug as a stable liquid solution. They include triglycerides, mono and diglycerides, lipophilic surfactants, hydrophilic surfactants and co solvents; excipients with a wide variety of
physicochemical properties. The main advantage of lipid formulation is that the drug could remain in solution throughout its period in the gastrointestinal track.

**Solid Lipid Nanoparticles (SLN)**

Solid lipid nanoparticles (SLN) are lipid based submicron particles in the range of 10-1000nm. Various types of nanoparticles used in biomedical research and drug delivery are inorganic nanoparticle, polymeric nanoparticle, solid lipid nanoparticle, liposome, nanocrystal, nano tube and dendrimer. The advantages of solid lipid nanoparticles are particularly those in the range of 120-200nm are not readily taken up by the cells of reticuloendothelial system (RES) and bypass liver and spleen filtration [35]. Controlled release of incorporated drug can be achieved up to several weeks [36]. Further by coating with or attaching ligands to SLN, there is an increased scope of drug targeting. SLN formulations are stable for even 3 years have been developed; this is of paramount importance with respect to other colloidal carrier systems [37] with high drug payload. Excellent reproducibility and feasibility of incorporating both hydrophilic and hydrophobic drugs can be attained. Various types of method are used to prepared solid lipid nanoparticles such as high pressure homogenization, ultrasonication method, microemulsion method, solvent emulsification, solvent evaporation and solvent injection method. Among all this method described above the microemulsion method shows thermodynamically stable, transparent, isotropic, low-viscous and colloidal dispersion. Microemulsions are clear or slightly bluish solutions being composed of a lipophilic phase (e.g. lipid), a surfactant and in most cases also a co-surfactant and water. The microemulsions show properties of real macroemulsions and simultaneously properties of a real solution. Addition of a microemulsion to water leads to precipitation of the lipid phase forming particles. To form a microemulsion with a lipid being solid at room temperature, the microemulsion needs to be produced at temperature above the melting point of the lipid. The lipid (fatty acids and/or glycerides) are melted, a mixture of water, co-surfactant(s) and the surfactant is heated to the same temperature as the lipid and added under mild stirring to the lipid melt. A transparent, thermodynamically stable system is formed when the compounds are mixed in the correct ratio for microemulsion formation. This microemulsion is then dispersed in a cold aqueous medium 38°C under mild mechanical mixing, thus ensuring that the small size of the
particles is due to the precipitation and not mechanically induced by a stirring process [38, 39].

1.6. Pharmacokinetics and bioavailability of HMP’s
HMP’s complex nature is a barrier for correlating the pharmacological activity hence, this major hurdle in developing in vitro reflects the obstacle for development of phytoconstituents into drugs [40]. In general, in vitro studies are easy to perform, cost effective and the significance of those observations depends upon the amount of drug available at the active site of action.

To associate phytoconstituents with pharmacological and clinical effects two aspects needed to be taken consideration as follows

- To determine bioavailability, which determines the rate of absorption of HMP’s in to systemic circulation after administration and
- To investigate the metabolic pathway, elimination pathway and pharmacokinetics. Chemical API’s have bio pharmaceutics and pharmacokinetics data available contrary to HMP’s because of their complex chemical entities.

However in the recent years research on bioavailability and pharmacokinetics of some HMP’s is made available due to availability of highly advanced sensitive and selective analytical instruments such as HPLC/MS/NMR and HPLC/MS/MS [41, 42]. Hence, with the aid of these instruments key findings such as starting ingredients used for the synthesis and preparation of those compounds were came to light. Depending on in vitro studies HMP’s can be categorized into different categories such as bioactive, co-active, interactive, toxic and also mutagenic. Determination of in vitro results of HMP’s reflects its comprehensive chemical profiling including pharmacokinetics which makes it accessible and available for converting them into a therapeutic agent. Moreover scientific investigation of HMP’s for these data will provide a platform for assessing therapeutic usage and curative claims world-wide. Certain regulatory authorities of various countries like EMEA, USFDA has set some guidelines for investigating safety, quality and efficacy of HMP’s in terms of NDA/IND (New drug application/Investigational new drug) process [43].
However, with all the above indications importance of determination of HMP’s bioavailability and pharmacokinetics can be understood to predict the full image of advantages for their therapeutic potential. Therefore, there is an urgent need for research to develop and investigate in-depth on the HMP’s which are therapeutically valuable and benefit the man-kind.