2. REVIEW OF LITERATURE

Quality control (QC) and standardisation of herbal extracts are very important to protect the integrity of herbal extracts for their pharmaceutical quality and safety. It also forms a part of prerequisite for the reproducibility of the effect of the active ingredient from one batch to another batch. QC and standardization of herbal drugs have been associated with many problems like identification and authentication of raw drugs, batch reproducibility, physico-chemical stability, adulteration and contamination etc. Apart from these issues, any herbal material and its extract, has hundreds of unknown components and many of them are in low amount. Moreover, there usually exists variability within the same herbal materials. Several international regulatory agencies such as WHO, USFDA and European Agency for evaluation of medicinal plants (EMEA) recommends such studies at various stages of herbal drug development. Therefore, this chapter covers an overview on some case examples of quality control, standardization, pharmacokinetics, approaches to overcome bioavailability issues of herbal drugs, and herb-drug interactions in the treatment of various chronic disorders. Further, this chapter also reviews the published data on the herbal drug under investigation; Gymnema sylvestre R.Br. and its biomarker; Gymnemagenin. The chapter is divided into six major sections mentioned below and is discussed in brief;

1. Quality control and modern standardization tools
2. Pharmacokinetics and bioavailability of natural products
3. Approaches to overcome poor bioavailability issues of herbal drugs
4. Herb-drug interactions
5. The herb under investigation: Gymnema sylvestre R.Br.(Plant Profile)
6. Profile of standard drugs and polymers

2.1 Quality control and modern standardization tools

In general, the methods for quality control of herbal medicines involve sensory (macroscopic and microscopic examinations) and analytical inspection using instrumental techniques such as, TLC, HPTLC, HPLC, GC-MS, and spectrophotometric analysis, etc. However, these chromatographic methods are still not sensitive or reproducible enough for detecting trace-level of parent natural products or their metabolites in biofluids. On the other hand, because many alkaloids,
flavonoids or sesquiterpenoids are very unstable and decompose fast in human body\textsuperscript{49, 50, 51} it is required to determine their metabolic fates including structure, characterization and quantitative analysis of metabolites. Many terpenoid saponins have a narrow therapeutic index with serious side effects\textsuperscript{52}, which makes it essential to accurately measure them in blood samples from a safety point of view. Since the introduction of new approaches of hyphenated chromatography and spectrometry such as high-performance liquid chromatography–diode array detection (HPLC–DAD), gas chromatography–mass spectroscopy (GC–MS), capillary electrophoresis–diode array detection (CE-DAD), HPLC–MS and HPLC–NMR provide the additional spectral information which becomes major contribution tool for quality control of herbal medicines\textsuperscript{53}. Here, it is important to highlight the feasibility and potential of HPLC coupled MS techniques in the identification and quantification of natural products (flavonoids, alkaloids, saponins and sesquiterpenoids) in biological fluids, using few examples of natural products. In most cases, while using HPLC-MS/MS as analytical tool, the biological samples cannot be assayed directly. Instead, they require a pretreatment to remove matrix components (endogenous proteins, carbohydrates, salts, and lipids) that might contaminate the system or cause ion suppression where high sensitivity is required. Protein precipitation technique (PPT), solid phase extraction (SPE) and liquid-liquid extraction (LLE) are the main sample preparation techniques used with HPLC–MS/MS to analyze natural products in biofluids. It is difficult to extract highly polar and hydrophilic natural products from plasma using organic solvents; therefore, PPT can be used in such cases. SPE is more preferred over PPT because of its high selectivity, speed of extraction, ability to reduce the serum background greatly, and it requires fewer amounts of organic solvents even in comparison with that of LLE\textsuperscript{54}. LLE is especially suitable for lipophilic compounds. Flavonoids in biofluids were usually extracted by ethyl acetate after acidification\textsuperscript{55,56} whereas alkaloids were usually extracted by chloroform or ether after alkalification\textsuperscript{57,58,59} Saponins and sesquiterpenoids were mainly extracted by n-butanol, methylene chloride, ether or ethyl acetate\textsuperscript{60, 61}. HPLC-MS/MS has been explored in multiple ways to study the behaviour of and to identify natural products in biological matrices. It is becoming crucial part of natural product chemistry and drug discovery. LC-MS has shown its attraction for supporting \textit{in vitro} and \textit{in vivo} studies of drug metabolic stability in which the levels of parent drug are quantitated at various time points\textsuperscript{62, 63}. Recently, LC-MS application has been expanded into characterizing
active component of traditional Chinese medicines as well\textsuperscript{64} (Table 1). Following
table describes few examples of natural products which has been characterized in
biological samples with the use of HPLC-ESI-MS/MS.

**Table 1. Overview on applicability of LC-MS methods of natural products**

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Biofluid</th>
<th>Extraction technique</th>
<th>Scan Mode</th>
<th>MS analyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hesperidin, Naringin,\textsuperscript{65} Neohesperidin, Naringenin and Hesperetin, flavanoids obtained from <em>Fractus aurantii</em> extract</td>
<td>Plasma</td>
<td>Solid Phase Extraction</td>
<td>+; ESI ; MRM</td>
<td>TQ</td>
</tr>
<tr>
<td>Tetrahydropalmatine\textsuperscript{66}, Protopine, and Palmatine, alkaloids obtained from <em>Rhizoma Corydalis decumbentis</em></td>
<td>Plasma</td>
<td>Liquid-Liquid Extraction</td>
<td>+; ESI ; SIM</td>
<td>SQ</td>
</tr>
<tr>
<td>Kakkalide isoflavone\textsuperscript{67} from dried flower of <em>Pueraria lobata</em> (Willd.)</td>
<td>Plasma</td>
<td>Solid Phase Extraction</td>
<td>-; ESI ; SIM</td>
<td>TQ</td>
</tr>
<tr>
<td>Gymnemagenin\textsuperscript{68}, triterpenoid saponin from <em>Gynnema sylvestre</em></td>
<td>Plasma</td>
<td>Liquid-Liquid Extraction</td>
<td>-; ESI ; MRM</td>
<td>TQ</td>
</tr>
<tr>
<td>Glycyrrhetic acid\textsuperscript{69}, a triterpenoid saponin from <em>Glycyrrhiza glabra</em></td>
<td>Plasma</td>
<td>Solid Phase Extraction</td>
<td>-; ESI ; SIM</td>
<td>SQ</td>
</tr>
<tr>
<td>Glycyrrhizin, saponin\textsuperscript{70} from <em>Glycyrrhiza glabra</em></td>
<td>Plasma</td>
<td>Liquid-Liquid and Solid Phase Extraction</td>
<td>+; ESI ; MRM</td>
<td>TQ</td>
</tr>
</tbody>
</table>


However, these LC-MS techniques, apart from natural products, have been applied to
a wide array of synthetic active pharmaceutical ingredients (API’s), such as anti-
cancer, anthelmintics, antibiotics, anxiolytics, analgesics, antiepileptics, specific
receptor antagonists, antioxidants, etc\textsuperscript{71}. LC-MS has also become most popular
analytical platform for qualitative and quantitative analysis of metabolites in
analytical chemistry, biochemistry, clinical studies and many other fields\textsuperscript{72}. With
modernization, the future generation LC-MS systems will be more robust and easier
to handle. Considering the recent developments in quadruple time of flight (Q-TOF) instruments, LC-MS would become a powerful tool in the determination of natural products, synthetic products and their metabolites in blood, plasma, serum, urine, bile or faeces. These analytical techniques have increased the attention of investigators because of their selectivity, specificity, speed and accuracy in pharmacokinetic analysis of biological samples. Under mentioned, is the review and few examples of developed pharmacokinetics of natural products using HPLC-MS/MS techniques. Further this review continue describing in brief about the importance of pharmacokinetic studies of natural products.

2.2 Pharmacokinetics and bioavailability of natural products

A general belief, that herbal drugs are safe and free from side effects, has gained much popularity in recent years with tremendous growth and usage of phytopharmaceuticals. Evidence-based verification of the efficacy of herbal medicinal products (HMP’s) is still lacking. However, in recent years, data on evaluation of the therapeutic and toxic activity of herbal medicinal products became available. Since long natural product scientists have been studying pharmacodynamics but, less attention has been paid on pharmacokinetics of plant extracts and their phytoconstituents. Unlike pharmaceuticals, pharmacokinetics of herbal products, mixture of known and unknown components, is always challenging due to their complexity, unavailability or inadequacy of standards and methods. Moreover, lack of pharmacokinetic studies is a biggest hindrance in the modernization of herbal products. Pharmacokinetic studies are of prime importance prior to clinical trials of herbal products to make, herbal remedies, evidence-based drugs. Pharmacokinetic study includes absorption, distribution, metabolism and excretion. Therapeutic outcome depends on the rate and extent at which drug reaches at the site of action, and its bioavailability. A better understanding of the pharmacokinetics and bioavailability of phytopharmaceuticals can help in designing rational dosage regimen; also help to understand the action of the body on the drug, which have numerous useful applications both in toxicology and biopharmaceutics. In this review, pharmacokinetic and bioavailability studies that have been conducted for some of the more important or widely used phytopharmaceuticals and extracts are critically evaluated.
St. John's Wort (*Hypericum perforatum*) extract has been known to have medicinal properties since antiquity. It has been cited in the works of Hippocrates, Dioscorides and the medieval herbal handbooks. M. Agrosi *et al.* in 2000 reported the bioavailability of main active principles of *Hypericum perforatum* extract, namely hypericin and hyperforin in humans, after a single oral administration of the extract in man in softgel capsules. Few other studies reported the pharmacokinetics of hypericin and oral bioavailability of hyperforin, using tablet formulations of hypericum extract. Pomegranate (*Punica granatum*) a traditional Chinese as well Indian medicine used as antibacterial, anti-inflammatory and hemostasis agent is rich of phenolic compounds. Extracts from many parts of this plant such as juices, seed oil and peel have been reported to exhibit strong antioxidant activity. Presence of phenolic nature, ellagic acid (EA) makes it more potent antioxidant and inhibitor of carcinogenicity and mutagenicity. Even after pharmacologically proved potential, the presence of EA was not observed at the tumour site. Although various metabolites of EA have been found in various biological matrices, no EA was found in plasma samples. Chinese research group conducted study for determination of pharmacokinetic properties of EA after oral administration of pomegranate leaf extract. The results showed that the level of EA in plasma was detectable and much higher than the concentration after oral administration of EA alone in comparison with that of pomegranate leaf extract according to other studies.

*Boswellia serrata* has been used in traditional medicine for the treatment of rheumatism, nervous system related diseases and for topical anti-inflammatory diseases since antiquity, however, human kinetic studies on extract have not been reported. Hence, to better elucidate its effects in humans and determine its optimal dosing, pharmacokinetic study on 11-β-boswellic acid constituent of *B. serrata* was conducted. No adverse effects were seen after administration of 333 mg as single dose of 11-β-boswellic acid. These parameters provided a base-line for further exploration of what the body does to the drug and the pharmacodynamic correlation. It was concluded that *Boswellia serrata* is a safe drug and well tolerated after oral administration.

*Andrographis paniculata* Nees is one of the most important medicinal plant, used in Indian, Chinese and Ayurvedic medicine for gastric disorders, colds, influenza and
other infectious diseases. The standardized extract of *A. paniculata* contains andrographolides and deoxyandrographolides which is called ‘Kan Jang’, used for treatment of common cold from last 20 years. Pharmacokinetic and oral bioavailability of andrographolides has been studied in rats, humans when administered in fixed dose as ‘Kan jang’. Pharmacokinetics of andrographolides was also established in rabbits after administration of aqueous extract of *A. paniculata*.

Ginseng (the root of *Panax ginseng* C.A.Meyer, family Araliaceae) is an orally administered medicine which has been frequently used in few Asian countries namely Korea, China and Japan. Ginseng is also one of the herbal medicinal remedies most commonly used by American consumers. Ginseng has been reported to have various biological effects including antiinflammatory activity and antitumor effects. The effects of ginseng are attributable to its major ginsenosides constituents. Absorption, metabolism and distribution study was carried out for ginseng in human subjects considering the individual differences in intestinal microbial flora and metabolic ability. This study could not reveal the complete excretion via metabolism and urination. Absorption of the final metabolites of ginseng is independent of the metabolite transforming activity of intestinal microflora but the $T_{\text{max}}$, $C_{\text{max}}$ and AUC of the transformed metabolites are dependent on the activity of each individual’s microbial flora.

*Glycyrrhiza glabra* (liquorice), has been used in the treatment for chronic hepatitis in Japan for more than 20 years. A number of components have been isolated from liquorice, including a water-soluble, biologically active complex that accounts for 40-50 percent of total dry material weight. Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of liquorice root. After oral administration of liquorice in humans, the main constituent, glycyrrhizic acid, is hydrolyzed to glycyrrhetic acid by intestinal bacteria possessing a specialized β-glucuronidase. Glycyrrhetic acid is 200-1000 times more potent inhibitor of 11-β-hydroxysteroid dehydrogenase (involved in corticosteroid metabolism) than glycyrrhizic acid; therefore, its pharmacokinetics after oral intake has been found more relevant.

Curcumin (diferuloyl methane) a polyphenol, is an active principle of the perennial herb *Curcuma longa* (commonly known as turmeric). The yellow-pigmented fraction
of turmeric contains curcuminoids, which are chemically related to its principal ingredient, curcumin. Traditionally, turmeric has been put to use as food stuff, cosmetic, and medicine and as spice. In ayurvedic medicine, curcumin is a well-documented medicine for various respiratory conditions (e.g., asthma, bronchial hyperactivity, and allergy) as well as for liver disorders, anorexia, rheumatism, diabetic wounds, runny nose, cough, and sinusitis. It has traditionally been used because of its good therapeutic effect, as an anti-inflammatory, and many of its therapeutic effects have been confirmed by modern scientific research such as anticarcinogenic\textsuperscript{92,93} antimicrobial\textsuperscript{94}, and hepatoprotective\textsuperscript{95}. The pharmacokinetics and pharmacodynamics of curcumin have been widely investigated. Perhaps the first study to examine the uptake, distribution, and excretion of curcumin was conducted in 1978 by Wahlstrom and Blennow in Sprague-Dawley rats. When administered orally at a dose of 1g/kg, approximately 75% of the ingested curcumin was excreted in the feces and only negligible amounts in the urine. As indicated by blood plasma levels and biliary excretion, curcumin was poorly absorbed from the gut. No apparent toxic effects were seen after doses of up to 5g/kg. When intravenously injected, curcumin was actively transported into the bile. Most of the drug was metabolized\textsuperscript{96}, however, again suggesting poor absorption and rapid metabolism. In another study in which 400 mg curcumin was administered orally to rats, most of the administered curcumin (40%) was excreted unchanged in the feces, none in the urine and none in heart blood. Intra peritoneal administration of curcumin at dose of 0.1mg/kg traces of the curcumin was detected in kidney, liver, and in brain\textsuperscript{97}. As most of these studies indicated, curcumin has poor bioavailability, several groups have investigated ways to enhance its bioavailability\textsuperscript{98}. Pharmacokinetic data is of particular interest so as to identify the bioavailability, to assess to what degree and how fast compounds are absorbed after administration of plant extracts and/or their phytoconstituents. Further it can help for elucidation of metabolic pathways (yielding potentially new active compounds), and to assess the elimination routes and their kinetics. These studies become an important issue to link data from pharmacological assays and clinical effects.

Apart from the above mentioned studies for pharmacokinetics of natural products few more examples where the pharmacokinetics has been reported (Table 2).

Department of Pharmacognosy, JSSCP (JSS University, Mysore), Udthagamandalam-643001

15 | P a g e
Table 2. Pharmacokinetics studies of natural products

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Name of the Plant extract/ phytoconstituent</th>
<th>Pharmacokinetic parameters observed</th>
<th>Biomarker used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethanolic extract of fruit of <em>Piper sarmentosum</em></td>
<td>Pellitorine, sarmentine; showed good oral bioavailability, tissue distribution, and both get excreted in urine as metabolites, whereas, sarmentosine excreted unchanged in feces and was not absorbed from the intestine.</td>
<td>Pellitorine, Sarmentine, Sarmentsone&lt;sup&gt;99&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanolic extract of <em>P.Ginseng berry</em></td>
<td>Ginsenoside Re showed very short half-life and low oral bioavailability.</td>
<td>Ginsenoside Re, Rg1 and Rh&lt;sup&gt;100&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.</td>
<td>Aqueous extract of <em>Paeoniae radix</em></td>
<td>Paeoniflorin showed higher binding activity to organs and lower blood distribution, the low bioavailability was also possible because of low paoniflorin concentration in plasma (about3%).</td>
<td>Paeoniflorin&lt;sup&gt;101&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.</td>
<td>Herbal preparation of <em>Swertia chirata</em></td>
<td>Pure markers were determined upto 48h after <em>i.v.</em> administration of 5mg/kg dose. The plasma concentration time profile of mangiferin and amarogentin was traceable up to 24h and 0.75h, respectively.</td>
<td>Mangiferin and amarogentin&lt;sup&gt;102&lt;/sup&gt;</td>
</tr>
<tr>
<td>5.</td>
<td><em>Rheum undulatum L.</em></td>
<td>The plasma rhein <em>C&lt;sub&gt;max&lt;/sub&gt;</em> reached between 1-2h after administration, and the concentrations decreased thereafter.</td>
<td>Rhein&lt;sup&gt;103&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.</td>
<td>Pinostrobin(PI) (5-hydroxy-7-methoxy flavanone,) is a natural flavonoid.</td>
<td>After intra gastric administration, the half-life (1/2) was 6.26±0.31h. The AUC curve of Pinostrobin after intragastric administration was 3817.80 ± 352.89 ng min/mL.</td>
<td>Pinostrobin&lt;sup&gt;104&lt;/sup&gt;</td>
</tr>
<tr>
<td>7.</td>
<td>Fuzi (lateral roots of <em>Aconitum carmichaeli</em>)</td>
<td>Results of multiple administration of aconitine showed mean plasma concentration time curves, <em>C&lt;sub&gt;max&lt;/sub&gt;</em>, MRT, and <em>t&lt;sub&gt;1/2&lt;/sub&gt;</em> had no significant variations when compared with those of single dose administration of Fuzi extract. The protein binding of aconitine was lower. The absolute bioavailability of aconitine was found low.</td>
<td>Aconitine&lt;sup&gt;105&lt;/sup&gt;</td>
</tr>
<tr>
<td>8.</td>
<td><em>Polygonum cuspidatum</em> extract</td>
<td>Emodin in the extract was rapidly absorbed after the extract exposure. The mean plasma emodin concentration occurred 24h after administration, emodin distributed extensively.</td>
<td>Emodin&lt;sup&gt;106&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
2.3 Approaches to overcome poor bioavailability issues of herbal drugs

As most of these pharmacokinetic studies have indicated, even though there are numerous therapeutic applications based on medicines of plant origin, poor oral bioavailability of bioactive remains major concern in herbal drug development. The poor bioavailability of bioactive constituents is due to low solubility, low penetration, GIT biotransformation, hepatic metabolism, high protein binding and renal or bile excretion. So, there is an urgent need to overcome this issue. To overcome this poor solubility or erratic bioavailability issue various approaches have been developed such as pharmaceutical salts, prodrugs, pH adjustments, complexation, emulsions, micellization/surfactant system, novel drug delivery approaches such as development of nano dosage forms (polymeric nanoparticles and nanocapsules, liposomes, solid lipid nanoparticles, phytosomes and nano-emulsion etc.) have a number of advantages for herbal drugs, including enhancement of solubility and bioavailability, protection from toxicity, enhancement of pharmacological activity, and enhancement of stability. This review highlights with few examples of novel drug delivery and other conventional approaches, used for plant extracts and phytoconstituents to overcome the bioavailability issues.

Dried fruits of *S. marianum* contain silymarin, a natural lipoprotic agent which has been reported for its low oral bioavailability. To overcome this issue, silymarin was encapsulated into liposomes for its buccal delivery. Liposomal buccal delivery of silymarin showed enhanced bioavailability with significant lower dose. In another standardized extract from the fruit seeds of *Silybum marianum* (L.) Gaertn. (*Milk thistle*, Asteraceae) was incorporated into self-emulsifying pellets in order to improve oral bioavailability of its active constituents.

<table>
<thead>
<tr>
<th>No.</th>
<th>Standardized leaf extract of <em>Ginkgo biloba</em></th>
<th>The mean plasma concentration–time profiles of ginkgolides A, B, C and bilobalide have been reported</th>
<th>Ginkgolides A, B, C and bilobalide</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Standardized <em>Epimedium</em> extract</td>
<td>All five prenylflavonoids exhibit non-linear dose-dependent increase in the area under concentration versus time curves. Two distinct pharmacokinetic patterns were evident, an early phase where inicaritin and icarisideII reached t&lt;sub&gt;max&lt;/sub&gt; at 0.5, 1h, respectively and a late phase where inicarisisdeI, icaritin and desmethylicaritin peaked at t&lt;sub&gt;max&lt;/sub&gt; 8h.</td>
<td>Prenylflavonoids which includes icarin, icarisideI, icarisideII, icaritin, and desmethylicaritin</td>
</tr>
</tbody>
</table>

107
108
109
110
111
112
Artemisinin (ART) and its derivatives are widely used throughout the world for its antimalarial property. ART is an endoperoxide-containing sesquiterpene developed from an ancient Chinese herbal remedy, which was used by Chinese herbal medicine practitioners for at least 2000 years. However, ART has many problems such as, low bioavailability due to its low solubility, metabolizes quickly in vivo and has an initial burst effect and high peak plasma concentrations. ART is not very stable and easily decomposes, most probably by the opening of the lactone ring, due to its unusual peroxy group, and crystal structure. It is difficult to disperse homogeneously because of their hydrophobicity in solution or blood; and a greater number of injections is necessary because of the short-duration effect. To overcome these problems nanocapsules with controlled-release system was developed so as to increase dissolution and improve release time\textsuperscript{113}.

Paclitaxel, a novel antineoplastic agent with unique molecular mechanism of action, is clinically active against advanced ovarian and breast cancer, and is under investigation for therapy of various cancers. Although it is therapeutically very effective, its poor biopharmaceutical properties such as poor solubility and permeability severely limit its clinical usage. Investigation was carried out to overcome this problem and nanoemulsion of paclitaxel was developed, which showed enhanced peroral bioavailability significantly to more than 70%. The developed formulation was safe and effective for both peroral and dermal delivery of paclitaxel\textsuperscript{114}.

Curcumin, the natural anticancer drug and its optimum potential is limited due to lack of solubility in aqueous solvent, degradation at alkaline pH and poor tissue absorption. In order to enhance its potency and improve bioavailability, synthesis of curcumin loaded nanoparticulate delivery system was developed. Nanoparticulate curcumin was more bioavailable and had a longer half-life than native curcumin as revealed from pharmacokinetics study\textsuperscript{115}.

A secoiridoid glycoside isolated from Indian medicinal plant \textit{Swertia chirata}, called amarogentin found increased efficacy for its antileishminial activity when delivered in the form of niosomes and liposomes in comparison with that of free amarogentin\textsuperscript{116}.

Silymarin, Curcumin, Green Tea, and Grape Seed Extracts were incorporated into phytosomes to overcome their bioavailability issue. Developed phytosomes showed markedly enhanced intestinal absorption and tissue delivery of specific polyphenols, also significantly improved efficacy compared to the chemically equivalent non-
phytosome form with improved efficacy and with no adverse reports\textsuperscript{117}. Similarly
developed novel approaches which are highly useful and find various advantages in
the delivery of herbal medicines are enlisted (Table 3). Apart from the developed
novel approaches, there are conventional methods which also have been used for
improving the solubility of phytoconstituents, which is further responsible for
improving its bioavailability and efficacy as well. Quercetin, one of the hydroxy-
flavones, for which after chemical reactions, mixture of mono-, di-, and tri-sodium
salts were prepared, showed improved free radical scavenging activity, with enhanced
solubility\textsuperscript{118}.

**Table 3.** Examples for novel approaches developed for phytoconstituents and extracts

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant Extract/Phytoconstituents</th>
<th>Novel Approach developed</th>
<th>Application of novel approach</th>
<th>Activity Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Curcumin</td>
<td>Nanoparticles</td>
<td>Specific targeted delivery by antibody coupled particles</td>
<td>Anticancer\textsuperscript{119}</td>
</tr>
<tr>
<td>2.</td>
<td>Colchicine</td>
<td>Niosomes</td>
<td>High encapsulation of colchicine with good stability, prolonged release &amp; less side effects</td>
<td>Cytotoxicity\textsuperscript{120}</td>
</tr>
</tbody>
</table>
| 3.      | Plant extract of *Sapindus*
emarginatus | Nanoparticles            | Nanosaponin induces dose-dependent cancer cell death with lower toxicity on normal cells.       | Cytotoxicity to cancer cell lines\textsuperscript{121} |
| 4.      | *Glycyrrhiza glabra* L. leaf
extract                           | Phytocomplexes           | GIT exposure enhances indirect improvement of bioavailability.                                 | Antioxidant, Anti-inflammatory, Anti-genotoxic\textsuperscript{122} |
| 5.      | Zedoary turmeric oil from *Rhizoma* Zedoariae | Microspheres            | Sustained release and higher bioavailability                                                   | Hepatoprotective, Anticancer\textsuperscript{123} |
| 6.      | Vincristine                     | Transfersomes            | Increases entrapment efficiency and skin permeation                                             | Anticancer\textsuperscript{124}    |
| 7.      | Matrine                         | Ethosomes                | Matrine ethosomes can help to increase the percutaneous permeation of matrine *in vitro* and improved the anti-inflammatory activity of matrine *in vivo* in rat skin. | Anti-inflammatory\textsuperscript{125} |

Pharmacokinetic information about herbal remedies is not widely available due to
several factors including lack of studies, and inadequate reporting. Researchers have
long studied the effect of herbal medicines on the body, but have paid less attention to
the effects of the body on herbal medicines. These terms distinguishes classical
pharmacology between pharmacodynamics and pharmacokinetics. Pharmacokinetic parameters include absorption, distribution, metabolism and excretion of its various components. Knowledge of herbal pharmacokinetics can provide valuable information to aid practitioners in prescribing herbs safely and effectively. It may also enable useful predictions to be made, for example regarding possible interactions between herbal remedies and conventional pharmaceuticals. Drug-drug interactions constitute the bulk of the conventional pharmacokinetic literature, but currently herb-drug interactions are taking center stage, both in the popular media and in terms of increasing physician awareness of the widespread and often undisclosed use of herbal medicines by their patients, and the potential for significant pharmacokinetic interaction between herbs and prescription pharmaceuticals. Following review briefly gives an idea about the basics of herb-drug interaction studies, its types with few examples of pharmacokinetic and pharmacodynamic reported interactions and its limitations.

2.4 Herb-drug interactions
The efficacy of drug therapy depends on many factors related to a drug’s pharmacokinetic and pharmacodynamic properties, which can be modified by differences in genetic polymorphisms, age, gender, circadian rhythms, intestinal bacteria, pathophysiological conditions, pharmaceutical dosage form and xenobiotics. One particular case is the co-administration of traditional drugs and herbal medicinal products (i.e. dietary supplements containing medicinal herbs or the herbal medicines traditionally used in phytotherapy for treating or preventing diseases), which may cause unexpected interactions. The high risk inherent to drug interactions is well known, though various studies have indicated that 14–31% of prescription drug users combine herbal products with traditional medicines\textsuperscript{126}. It has been documented that as many as 31% of patients use herbal supplements concurrently with prescribed drugs and 70% of them do not report the use of these products to their physician. Thus, there is an increase in possibility for herb-drug interactions leading to beneficial, sub-therapeutic, toxic or sometime may be fatal clinical outcome of the therapy\textsuperscript{127}.

2.4.1 Pharmacodynamic interactions
Pharmacodynamic interactions involve interactions of conventional drugs with herbs for the same targets such as enzymes or receptors leading to additive, synergistic,
antagonistic effects. It has been considered that herb contains thousands of compounds with diverse chemical nature and therefore, they have different affinities towards these primary and/or secondary therapeutic targets (Figure 3). The probability of possible interaction would be beneficial or unwanted. There are several pharmacodynamic interaction studies have been documented based on animal studies, clinical trials and case reports for conventional drugs used in various chronic diseases and are mentioned in the (Table 4).

![Figure 3. Schematic representation of pharmacodynamic herb-drug interactions](image)

*Ginkgo biloba* is a traditional Chinese and Japanese herbal medicine used mainly for memory loss, Alzheimer’s disease and circulatory disorders and has been prescribed along with antipsychotic drugs. Pharmacodynamic interaction of *Ginkgo biloba* extract (EGb) and classical antipsychotic drug, haloperidol has been studied in chronic schizophrenic patient. EGb is reported to enhance the efficiency and reduce the extra pyramidal adverse effects of haloperidol leading to synergistic effect.

Pharmacodynamic interaction of Garlic (*Allium sativum*) with warfarin, an anticoagulant, has been reported for additive effect on coagulation mechanism suggesting associated risk for such combination. Another study on curry containing garlic and karela showed additive anti-diabetic activity when taken with oral antihyperglycemics like chlorpropamide, sulfonylurea derivatives. The interaction between *Hypericum perforatum* (St. John’s wort) and warfarin had been identified from spontaneous case reports. Seven cases of decreased warfarin effect following St. John’s wort treatment were reported by the Swedish Medical Products Agency. Between 1998 and 2000, 22 case reports of interactions with warfarin had been
reported to regulatory authorities in Europe\textsuperscript{133}. These interactions resulted in unstable INR values, most commonly with a decrease in INR value being observed. Concomitant intake of St. John’s wort was associated with loss of anticoagulant activity. Anticoagulant activity was restored when St. John’s wort treatment was terminated or the warfarin dose was increased. \textit{Piper methysticum} (Kava) is a drink widely used for its calming and tranquilizing properties by the native population in the islands of the South Pacific\textsuperscript{134}. Kava had been reported for its dopamine antagonism activity and cases of patients developing clinical signs suggestive of central dopaminergic antagonist have been described\textsuperscript{135}. The dopamine antagonistic properties of kava could explain the increase in the duration and number of ‘off’ periods in a patient with Parkinson’s treated concomitantly with levodopa. \textit{Glycyrrhiza glabra} (liquorice) is a common herb in Chinese traditional medicine and a component in Japanese herbal medicines such as preparations Xiao Chai Hu Tang (Shosaiko-to in Japanese). In a crossover study in healthy volunteers, oral administration of liquorice extract for 7 days did not significantly alter the pharmacokinetic and sedative effects of midazolam\textsuperscript{136}.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Sr. No & Herb & Drug/s & Effect & Mechanism & Risk/benefit & Study model \\
\hline
1. & \textit{Ginkgo biloba} & Haloperidol & $\uparrow$ efficiency & synergistic & Beneficial & Clinical trial \\
2. & \textit{Allium sativum} & Warfarin & $\uparrow$ clotting time & additive & Risk & Case report \\
 & & Chlorpropamide & $\uparrow$ in anti-diabetic effect & additive & Beneficial & Case report \\
3. & \textit{Hypericum perforatum} & Warfarin & $\downarrow$ in INR values & antagonistic & Risk & Case report \\
4. & \textit{Piper Methysticum} & Levodopa & dopamine antagonist & antagonistic & Risk & Case report \\
5. & \textit{Glycyrrhiza glabra} & Midazolam & no change in sedative effect & no effect & No risk/benefit & Healthy subject \\
\hline
\end{tabular}
\caption{Examples of pharmacodynamic interactions}
\end{table}

↑ indicates increase and ↓ indicates decrease in activity

\subsection*{2.4.2 Pharmacokinetic interactions}

Concomitant administration of herbs with conventional drugs affects absorption, distribution, metabolism and excretion (ADME) leading to toxic or sub-therapeutic
effect of the drug (Figure 4). Herbs affect the ADME of conventional drug upon co-administration. Herb modulates transporter proteins such as p-glycoprotein (an efflux protein) and/or organic anionic (OATP) as well as cationic transporter proteins (OCTP) required for transport of conventional drug present in gut, liver, kidney, brain, thus affecting the pharmacokinetic of co-administered drugs.

![Figure 4. Schematic representation of pharmacokinetic herb-drug interactions](image)

Herbal drugs also has modulatory effect on several metabolizing enzymes including CYP P450 (e.g. CYP 3A4, 2C9, 2C19; 2D6 and 2B6 etc.) which act as receptors and required for metabolism of conventional drug, leading to pharmacokinetic interaction when co-administered with conventional drugs. There are various pharmacokinetic interaction studies documented based on animal models, clinical trials and case reports for conventional drugs used in various chronic diseases (Table 5).
Table 5. Examples of pharmacokinetic interactions

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Herb</th>
<th>Drug/s</th>
<th>Effect</th>
<th>Mechanism</th>
<th>Study model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Plantago ovata</td>
<td>Carbamazepine</td>
<td>↓ absorption</td>
<td>high mucilage</td>
<td>Healthy volunteer</td>
</tr>
<tr>
<td>2.</td>
<td>Rheum palmatum</td>
<td>Digoxin</td>
<td>↓ absorption</td>
<td>cause diarrhea</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Filipendula ulmaria &amp; Salix nigra</td>
<td>Warfarin</td>
<td>affect distribution</td>
<td>displace protein binding</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Hypericum perforatum</td>
<td>Digoxin</td>
<td>affect absorption &amp; distribution</td>
<td>induction of p-gp</td>
<td>Healthy volunteer</td>
</tr>
<tr>
<td>4.</td>
<td>Hypericum perforatum</td>
<td>Cyclosporine</td>
<td>↑ metabolism</td>
<td>induction of p-gp and CYP 3A4</td>
<td>Case report</td>
</tr>
<tr>
<td></td>
<td>Theophylline</td>
<td>↑ metabolism</td>
<td>induction of CYP1A1 and CYP1A2</td>
<td>Case report</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Piper nigrum &amp; Piper longum (piperine)</td>
<td>Propranolol</td>
<td>↓ metabolism</td>
<td>Inhibition of CYP1A1,CYP1A2 and CYP2D6</td>
<td>Healthy volunteer</td>
</tr>
<tr>
<td></td>
<td>Theophylline</td>
<td>↓ metabolism</td>
<td>Inhibition of CYP1A1 and CYP1A2</td>
<td>Healthy volunteer</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Glycyrrhiza uralensis</td>
<td>Methotrexate</td>
<td>↓ elimination</td>
<td>MRP inhibition or substrate for OATP</td>
<td>Rat</td>
</tr>
<tr>
<td>7</td>
<td>Hydrastis canadensis</td>
<td>Indinavir</td>
<td>no change in ADME</td>
<td>-</td>
<td>Healthy volunteer</td>
</tr>
<tr>
<td>8</td>
<td>Pueraria lobata</td>
<td>Methotrexate</td>
<td>↓ elimination</td>
<td>MRP inhibition or substrate for OATP</td>
<td>Rat</td>
</tr>
<tr>
<td>9</td>
<td>Ginkgo biloba extract</td>
<td>Theophylline</td>
<td>↑ elimination</td>
<td>GBE pre-treatment increased CYP1A2 metabolic activity</td>
<td>Rat</td>
</tr>
</tbody>
</table>

↑ indicates increase and ↓ indicates decrease in activity

2.4.3 Absorption

Herbs that have hydro-colloidal carbohydrate components such as gums and mucilages are soluble in water but poorly absorbable; examples include psyllium, rhubarb, flaxseed, marshmallow, and aloe. These compounds are apt to bind to other drugs, particularly when consumed in their whole or powdered forms. For example, psyllium (an herb with high mucilage content) inhibits the absorption of
carbamazepine\textsuperscript{137}. Rhubarb and aloe can cause diarrhea, which reduces the action of drugs that have a narrow therapeutic index (eg, digoxin, warfarin).

### 2.4.4 Distribution
Herbs such as *Filipendula ulmaria* (meadow sweet) and *Salix nigra* (black willow), which contain pain-reducing salicylates, may displace highly protein-bound drugs such as warfarin and carbamazepine, thus increasing the adverse effects of the drugs\textsuperscript{138,139}. These products should not be taken concurrently. A single-blind, placebo controlled parallel study involving 25 healthy volunteers indicated that repeated intake of the *Hypericum perforatum* at 900 mg/day for 10 days resulted in a 25\% decrease AUC, digoxin concentration at the end of a dosing interval by 26\%, with the more pronounced decline in trough concentration as the duration of *H. perforatum* intake increased\textsuperscript{140}.

### 2.4.5 Metabolism
Drug metabolism is the modification or degradation of drugs. Metabolism can make drugs more or less toxic, active or inactive, or more easily eliminated from the body\textsuperscript{141}. The primary organ involved in metabolism is the liver, although metabolism has been documented in the kidneys, lungs, gastrointestinal system, blood, and other tissues\textsuperscript{142}. The most extensively studied family of isoenzymes found in the liver and gastrointestinal tract is the cytochrome P450 (CYP) system\textsuperscript{143}. There are many subunits of P450. Herbs can inhibit (decrease) metabolism, induce (increase) metabolism, or have no effect on each CYP450 isoenzyme subset. For example *Hypericum perforatum* decreases plasma concentrations (AUC) of several drugs including cyclosporine, digoxin and theophylline\textsuperscript{144}. Few case reports on interaction of *H. perforatum* and cyclosporine in heart, kidney, liver and pancreas transplant patients have been published\textsuperscript{145,146, 147}. Decreased blood trough concentrations of cyclosporine had been observed and associated with transplant graft rejection. The decrease in cyclosporine concentrations ranged from 25\% to 62\%\textsuperscript{148,149}. Some of the patients recovered spontaneously after stopping the herbal medicine, while others needed an increased cyclosporine dose. The fundamental mechanism for the interaction suggested the induction of p-gp and CYP 3A4 isoenzyme.
Another case report had been published on *H. perforatum* and theophylline interaction\(^{150}\). *H. perforatum* lowered plasma concentration of theophylline and when discontinued the concentration was doubled. These observations suggest that the intake of *H. perforatum* enhances the metabolism of theophylline. Theophylline is primarily metabolized by CYP1A2, implying that *H. perforatum* induces the expression of this enzyme in the liver.

*Piper nigrum* Linn (black pepper) and *Piper longum* Linn (long pepper) are spices used in traditional anti-diarrhoeal formulations. Piperine, a pungent alkaloid; is a major constituent in *P. nigrum* reported as bioenhancer for several drugs such as amoxicillin, cefotaxime\(^{151}\), nimesulide\(^{152}\), pentobarbital\(^{153}\) and curcumin\(^{154}\). The underlying mechanism as a bioenhancer is suggested through inhibition of metabolism (through several CYPs) or transporter protein such as p-gp. In a crossover study involving six healthy volunteers, treatment of piperine 20mg daily for 7 days followed by a single dose of propranolol (40mg) resulted in increased C\(_{\text{max}}\) and AUC\(^{155}\). Propranolol is mainly metabolized by CYP1A1, CYP1A2 and CYP2D6\(^{156}\). Inhibition of these two enzymes resulted in increased C\(_{\text{max}}\) and AUC of propranolol. Another study showed treatment with piperine 20 mg daily for 7 days followed by a single dose of theophylline (150mg) in healthy volunteers resulted in increased C\(_{\text{max}}\), AUC and decrease in t\(_{1/2}\)\(^{157}\). Theophylline is mainly metabolised by CYP1A1 and CYP1A2\(^{158}\). The altered pharmacokinetics of theophylline is considered to be due to inhibition of these CYP isoforms.

### 2.4.6 Elimination

Elimination is the process of removal of a drug from the body. The major organs involved in elimination are the kidney and liver, although other bodily processes, including saliva, sweat, or exhaled air, may be other pathways for elimination. Elimination through the liver is primarily through bile. There are not many true drug-drug or herb-drug interactions through bile elimination. Renal drug-drug interactions are dependent on the pH of the urine and the pH of the drug or on competition for the same pathway of elimination. If the pH of the urine and the drug are the same, renal reabsorption of the drug will be increased. When two drugs compete for elimination through a single route, one drug may competitively inhibit the elimination of the other. For examples, one of the studies on pharmacokinetic interaction of methotrexate (MTX) with liquorice (root of *Glycyrrhiza uralensis*) and isolated
component; glycyrrhizin showed significant increase in AUC and mean resistance
time (MRT) of MTX when administered concomitantly. The speculated mechanism
suggested that *G. uralensis* and glycyrrhetic acid, aglycone moiety of glycyrrhizin
inhibits BCRP (breast cancer resistance protein) and multi resistance protein (MRP)
and delays in MTX elimination or acts as substrates for organic anionic transporter
protein (OATPs)\(^{159}\). Another life threatening interaction study with *Pueraria lobata*
root decoction showed more than 50% mortality of animals with significant increase
in AUC and MRT of MTX\(^{160}\). The study suggested that underline mechanism could
be delays in MTX elimination because of inhibition of MRP or acts as substrate for
OATP.

2.4.7 Limitations of herb-drug interaction studies

Much of the available information about the interaction between herbal products and
prescribed drugs is obtained from case reports, although clinical studies are now also
beginning to appear in the literature. The published case reports are often incomplete
as they do not allow us to conclude that a causal relationship exists. Even documented
case reports have to be interpreted with great caution, as causality is not usually
established beyond reasonable doubt. According to the scoring system described by
Fugh-Berman and Ernst, 68.5% of the cases reported were classified as ‘unevaluable’
(i.e. reports contained inadequate information to assess the likelihood of an
interaction), 18.5% were classified as ‘possible’ (i.e. reports provided some evidence
for an interaction, but there may be other causes of the event) and 13% as ‘well
documented’ (reports appeared to provide reliable evidence for an interaction)\(^{161}\).

There is evidence that taking herbal preparations can result in pharmacokinetic or
pharmacodynamic interactions that represent a potential risk to patients taking
conventional medicines. The majority of interactions identified to date involve
medicines that require regular monitoring of blood levels (e.g. warfarin,
cyclosporine). Although some herb-drug interactions may be clinically insignificant,
(e.g. interaction between gum guar and penicillin V), but others may have serious
consequences (e.g. interaction between St John’s wort and cyclosporine)\(^{162}\). Knowing
that millions of patients take herbal and conventional medicines concomitantly, often
without the knowledge of their physicians, and considering the present lack of
understanding of herb–drug interactions, more research into this area seems a matter
of urgency.
2.5 PLANT PROFILE

*Gymnema sylvestre R. Br.*


**Classification**

- **Kingdom**: Plantae
- **Division**: Angiospermae
- **Class**: Dicotyledoneae
- **Order**: Contortae
- **Family**: Asclepiadaceae
- **Genus**: Gymnema
- **Species**: *sylvestre* R.Br.

**Vernacular names**

- **English**: Periploca of the woods
- **Hindi**: Gudmar
- **Sanskrit**: Mesasrngi
- **Tamil**: Sirukurunkay

**Botanical description**: Large climbers, rooting at nodes, leaves elliptic, acuminate, base acute to acuminate, glabrous above sparsely or densely tomentose beneath; Flowers small, in axillary and lateral umbel like cymes, Pedicels long; Calyx-lobes long, ovate, obtuse, pubescent; Corolla pale yellow campanulate, valvate, corona single, with 5 fleshy scales. Scales adnate to throat of corolla tube between lobes; Anther connective produced into a membranous tip, pollinia 2, erect, Carpels 2, unilocular; locules many ovuled; Follicle long, fusiform163.
2.5.2 Macroscopic Characteristics
Leaves of *G. sylvestre* are green in colour and stem is hairy and light brown. Leaf is 2-6 cm in length and 1-4 cm in width. The leaves are simple, petiolate, rounded to cordate base, margin entire, opposite with acute apex, reticulate venation, pubescent on both the surfaces. The odour is characteristic and taste of leaf is slightly bitter and astringent. It also possesses remarkable property of paralyzing the sense of the taste for sweet substances for few hours\(^{164, 165}\).

2.5.3 Traditional uses
Sushruta describes *Gymnema sylvestre*, as a destroyer of madhumeha (glycosuria) and other urinary disorders. On account of its property of abolishing the taste of sugar it has been given the name of gur-mar meaning sugar destroying and it is believed therefore that it might neutralize the excess of sugar present in the body in Diabetes mellitus\(^{166}\). The plant is also reported to be bitter, astringent, acrid, thermogenic, anti-inflammatory, anodyne, digestive, liver tonic, emetic, diuretic, stomachic, stimulant, anthelmentic, laxative, cardiotonic, expectorant, antipyretic and uterine tonic. It is useful in dyspepsia, constipation, jaundice, haemorrhoids, renal and vesical calculi, cardiopathy, asthma, bronchitis, amenorrhoea, conjunctivitis and leucoderma\(^{167, 168}\).

2.5.4 Pharmacological Review

**Anticancer activity**
The effects of chloroform, ethyl acetate and alcoholic extracts of *Gymnema sylvestre* were tested on A549 (epithelial cells of human lung cancer), cell lines and MCF7 (epithelial cells of human breast cancer, Soule et al,1973) cell lines *invitro* by MTT assay method. All the three extracts have shown concentration dependent activity and the IC\(_{50}\) values were almost similar. However the effect on A549 cells was not prominent. The chloroform and ethyl acetate extracts have shown comparatively better effect than alcoholic extract on A549 cell lines\(^{169}\).

**Leishmanicidal activity**
Leishmanicidal activity of saponin gymnemagenol isolated from leaves of *Gymnema sylvestre* was evaluated against leishmanial parasites. Gymnemagenol was found effective in inhibiting the growth of *L. major* promastigote, however it was not effective against *L. tropica*, promastigote, *L. aethiopica* even at higher concentrations\(^{170}\).
Antimicrobial activity
The ethanolic extract of Gymnema sylvestre leaves demonstrated antimicrobial activity against Bacillus pumilis, B. subtilis, Pseudomonas aeruginosa and Staphylococcus aureus and activity against Proteus vulgaris and Escherichia coli\textsuperscript{171}.

Antihyperlipidemic activity
Effect of hydro alcoholic extract of Gymnema sylvestre R.Br. leaf extract was studied at a dose of 200mg/kg on high fed with high cholesterol diet rats for seven days for its antihyperlipidemic potential. Gymnema sylvestre extract showed significant antihyperlipidemic activity which may be due to presence of flavanoids, phenols, tannins, and triterpenoids found in preliminary phytochemical screening\textsuperscript{172}.

Anti-inflammatory activity
The aqueous extract of G. sylvestre leaves was investigated for evaluation of anti inflammatory activity in rats at a dose 200, 300 and 500 mg/kg in carrageenin-induced paw oedema and cotton pellet method using phenylbutazone as standard. The aqueous extract at 300 mg/kg dose decreased the paw oedema volume by 48.5% within 4 h after administration, while the standard drug phenylbutazone decreased the paw oedema volume by 57.6% when compared with the paw oedema volume of control. The aqueous extract at the dose of 200 mg/kg and 300 mg/kg produced significant reduction in granuloma weight, when compared to control group\textsuperscript{173}.

Free radical scavenging activity
Antioxidant potential of aqueous extract of gymnema was evaluated in vitro against DPPH radicals and LDL oxidation. Results were found significant.

Antihyperglycaemic activity
Gymnema sylvestre a plant used in the Ayurvedic medicine of India for the treatment of diabetes mellitus has been known from antiquity and also to have an antisaccharin taste effect. The active principles are glycosides (several gymnemic acids) which shows selective anaesthetic effect\textsuperscript{174}. Experimental studies were conducted on rats fed on high carbohydrate diet for 15 days and later rendered hyperglycemic by injecting anterior pituitary extract 100mg/kg subcutaneously daily for ten days. These animals were treated with ethanol extract of Gymnema sylvestre at a dose of 100mg/kg orally. Results indicated insignificant reduction in blood sugar in normal rats, whereas
significant reduction in anterior pituitary treated hyperglycaemic rats. Effect of the drug was comparable to that of tolbutamide (50mg/kg) in the hyperglycaemic rats. The drug influenced the disturbed carbohydrate metabolism in hyperglycaemic animals by limiting the carbohydrate turnover and thus inhibiting the vicious cycle of hyperglycaemia\textsuperscript{175}. The inhibitory effects of an extract of \textit{Gymnema sylvestre} and purified gymnemic acids on gastric Inhibitory Peptide (GIP) release were studied in rats. The GIP release into the portal vein was in response to duodenal infusion of D-glucose in presence of leaf extract of \textit{Gymnema sylvestre} at a dosage of 0.5ml/kg. The results suggested that a glucose receptor interacted with the leaf extracts of \textit{Gymnema sylvestre} and purified Gymnemic acid. The inhibition of GIP release by Gymnemic acids observed was attributed to the interaction with the glucose receptor for GIP release which was similar in specificity to the active glucose transport system\textsuperscript{176}.

Water soluble fractions (GS3, GS4) of \textit{Gymnema sylvestre} were evaluated against streptozotocin induced diabetes for their effect on blood glucose homeostasis and pancreatic endocrine tissue. In the diabetic rats, fasting blood glucose level returned to normal after 60 days of GS3 and after 20 days of GS4 oral administration. In diabetic rat pancreas, both GS3 and GS4 doubled the islet number and beta cell number. This herbal therapy appeared to bring about blood glucose homeostasis through increased serum insulin levels provided by repair/regeneration of the endocrine pancreas\textsuperscript{177}.

2.5.5 Phytochemistry

\textit{G. sylvestre} leaves contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarene saponins are gymnemosides. The major bioactive constituents of \textit{Gymnema sylvestre} are a group of oleanane type triterpenoid saponins known as “gymnemic acids” \textbf{(Figure 6)} The latter contain several acylated (tigloyl, methylbutyroyl etc..) derivatives of deacetylgymnemic acid (DAGA) which is 3-O-β-glucuronide of gymnemagenin (3β, 16β, 21β, 22α, 23, 28-hexahydroxy-olean-12-ene). The individual gymnemic acids (saponins) include gymnemic acids I-VII, gymnemosides A-F, gymnemasaponins etc\textsuperscript{178,179,180}. Besides six known gymnemic acids, four new triterpenoid saponins, gymnemasins A, B, C and D, were isolated from the leaves of \textit{Gymnema sylvestre}. The aglycone, gymnemanol, which is a new compound, was characterized as 3, 16, 22, 23, 28-pentahydroxyolean-12-ene\textsuperscript{181}. 


Figure 6. Structures of gymnemic acids I-V, gymnemoside A, B and gymmemagenin.
2.6 REFERNCE DRUGS PROFILE

2.6.1 Glimepiride
Glimepiride belongs to second-generation sulfonyl urea class which is efficacious, generally well-tolerated, cost-effective option for the medical management of diabetes. Many comparative and non-comparative studies have evaluated the efficacy and safety of the second-generation sulfonylureas in patients with Type 2 diabetes.\textsuperscript{182}

Chemical Structure

![Figure 7. Structure of Glimepiride](image)

Solubility
Slightly soluble in methanol, sparingly soluble in methylene chloride, soluble in N, N-dimethylformamide, practically insoluble in water.

Therapeutic uses
Several possible explanations may account for a lower incidence of hypoglycemia seen with glimepiride. One is lower binding affinity with glimepiride than with the other sulfonyl ureas at the receptor site\textsuperscript{183}. In animals, glimepiride, as well as the other sulfonylureas, produces a biphasic pattern of insulin secretion characterized by a quick and short initial peak, followed by a longer sustained phase\textsuperscript{184}. The initial phase has been shown to be quicker and shorter with glimepiride than with glyburide; in addition, less insulin is secreted in response to a drop in glucose levels\textsuperscript{185}. This feature of a lower incidence of hypoglycemia makes glimepiride suitable for treating patients who are prone to hypoglycemia or who demonstrate renal impairment. It is also of benefit for patients in whom episodes of hypoglycemia could be particularly dangerous, such as the elderly\textsuperscript{186}.

Pharmacokinetic properties
Absorption
The bioavailability of glimepiride after oral administration is complete. Food intake has no relevant influence on absorption, only absorption rate is slightly diminished.
Maximum serum concentration ($C_{\text{max}}$) reaches approx. 2.5 hours after oral intake (mean 0.3 µg/ml during multiple dosing of 4 mg daily) and there is a linear relationship between dose and both $C_{\text{max}}$ and AUC (area under the time/concentration curve).

**Distribution**
Glimepiride has a very low distribution volume (approx. 8.8 litres), which is roughly equal to the albumin distribution space, high protein binding (>99%), and a low clearance (apprx. 48 ml/min). In animals, glimepiride is excreted in milk. Glimepiride is transferred to the placenta. Passage of the blood brain barrier is low\(^{187}\).

**Metabolism**
Glimepiride is completely metabolized by oxidative biotransformation after either an iv or oral dose. The major metabolites are the cyclohexyl hydroxy methyl derivative (M1) and the carboxyl derivative (M2). Cytochrome P450 II C9 has been shown to be involved in the biotransformation of glimepiride to M1. M1 is further metabolized to M2 by one or several cytosolic enzymes. M1, but not M2, possesses about 1/3 of the pharmacological activity as compared to its parent in an animal model; however, whether the meaningfulness of glucose-lowering effect of M1 is clinically is not clear\(^{188}\).

**Excretion**
When radiolabelled $^{14}$C-glimepiride was given orally, approximately 60% of the total radioactivity was recovered in the urine in 7 days and M1 (predominant) and M2 accounted for 80-90% of that recovered in the urine. Approximately 40% of the total radioactivity was recovered in faeces and M1 and M2 (predominant) accounted for about 70% of that recovered in faeces. No parent drug was recovered from urine or faeces. After iv dosing in patients, no significant biliary excretion of glimepiride or its M1 metabolite has been observed.

**Herb Drug Interactions**
The research reported for the study which was carried out to assess the protective potential of the synthetic sulfonylurea drug glimepiride and *Nerium oleander* plant extract on lipid profile, body growth rate, and renal function in streptozotocin-induced
diabetic rats. Treatment of diabetic rats with glimepiride and Nerium oleander extract offered protection in terms of lipid profile, growth rate and renal function, indicating their antidiabetic potential in human diabetes\textsuperscript{189}.

2.6.2 Metformin

Metformin (MET) is an oral anti-diabetic drug of the biguanide class. It is the drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function\textsuperscript{190}. It has been reported to be effective along with insulin in type 1 diabetes\textsuperscript{191}.

Chemical structure

\begin{center}
\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{metformin_structure.png}
\caption{Structure of Metformin}
\end{figure}
\end{center}

**Chemical Name:** N,N-dimethylimidodicarbonimidic diamide

**Solubility**

Freely soluble in water, slightly soluble in alcohol, insoluble in acetone, chloroform and in ether.

**Therapeutic uses**

MET is primarily used for type 2 diabetes, however, it is increasingly being used in polycystic ovary syndrome (PCOS)\textsuperscript{192}, non-alcoholic fatty liver disease (NAFLD)\textsuperscript{193} and premature puberty\textsuperscript{194}, these indications are still considered experimental. The benefit of metformin in NAFLD has not been extensively studied and may be only temporary although some randomized controlled trials have found significant improvement with its use.

**Pharmacokinetics**

**Absorption**

MET has an oral bioavailability of 33–55\% under fasting conditions, and is absorbed slowly\textsuperscript{195}. $C_{\text{max}}$ has been achieved within one to three hours after taking immediate-release metformin and four to eight hours with extended-release formulations\textsuperscript{196}.
Distribution
The plasma protein binding of metformin is negligible, as reflected by its very high apparent volume of distribution (300–1000 L after a single dose)\(^{197}\). Steady state is usually reached in one or two days. MET has acid dissociation constant values (pKa) of 2.8 and 11.5 and, therefore, exists very largely as the hydrophilic cationic species at physiological pH values. The metformin acid dissociation constant values (pKa) make metformin a stronger base than most other basic drugs with less than 0.01% unionized in blood. Furthermore, the lipid solubility of the unionized species is slight as shown by its low log P value of -1.43. These chemical parameters indicate low lipophilicity and, consequently, slow passive diffusion of metformin through cell membranes is unlikely.

Metabolism and Excretion
MET is considered as hard drug and not get metabolized in the body. Few report show that it slightly metabolized by CYP 2C11, 2D1 and 3A1/2\(^{198}\). MET has negligible plasma protein binding capacity and it undergoes active renal tubular excretion by organic cation transporter (OCT2)\(^{199}\). The average elimination half-life in plasma is 6.2 hours.

Herb-drug interactions
MET has been reported for its interaction with cimetidine in human. Results suggested that cimetidine significantly increased the MET AUC by 50% and reduced its renal clearance over 24 h by 27%. The mechanism suggested that cimetidine inhibits the renal tubular secretion of MET, resulting in higher circulating plasma concentrations\(^{200}\). One study on Ginkgo biloba standardized extract in normal as well type 2 diabetic patients has been reported. The study concluded that the co-ingestion of extract and MET did not significantly affect pharmacokinetic properties of MET\(^{201}\). The preclinical reports are available on kinetic interaction with isolated bacterial lipopolysaccharide\(^{202,203}\). The iv administration of lipopolysaccharide showed significant increase in AUC and decrease in clearance of MET. The mechanism suggested that the lipopolysaccharide decreases the expression of metabolizing enzymes, CYP 2C11 and 3A1/2 leading to increase in plasma MET concentrations. The study was conducted for investigation of antidiabetic activity of the various combinations of metformin (50 mg/kg) with aqueous extracts of the leaves of
Vernonia amygdalina (100 mg/kg). The ratios of extract to MET; 1:1, 2:1 and 1:2 (p.o.) were given to both normoglycemic and alloxan-induced diabetic Wister rats. The hypoglycemic effect of the combined agents suggested that their antidiabetic activities are addictive suggesting that the extract and metformin are acting through the same mechanism.

### 2.7 PROFILE OF POLYMERS

#### 2.7.1 Chitosan

The history of chitosan dates back to the 19th century, when Rouget discussed the deacetylated forms of the parent chitin natural polymer in 1859. During the past 20 years, a substantial amount of work has been reported on chitosan and its potential use in various bio-applications. Chitosan, poly-β (1→4)-2-amino-2-deoxy-D-glucose, is obtained by deacetylation of chitin, poly-N-acetyl-D-glucosamine, which occurs widely in lower animals, fungi etc. Most common commercial sources of chitin are exoskeletons of cabs and shrimps. Chitosan is obtained by thermo chemical deacetylation of chitin in the presence of alkali and naturally it occurs only in certain fungi (Mucoraceae).

**Chemical structure**

![Figure 9. Structure of Chitosan](image)

Chitosan molecule is a copolymer composed of N-acetyl-D-glucosamine and D-glucosamine units available in different grades depending upon the degree of acetylated moieties. It is a polycationic polymer that has one amino group and two hydroxyl groups in the repeating glucosidic residue. The carbohydrate backbone is very similar to cellulose, which consists of β-1,4-linked D-glucosamine with a variable degree of N-acetylation, except that the acetylamino group replaces the hydroxyl group on the C2 position. Thus, chitosan is a copolymer consisting of N-acetyl-2-amino-2-deoxy-D-glucopyranos & 2-amino-2-deoxy-D-glucopyranose, where the two types of repeating units are linked by β (1→4)-glycosidic bonds.
After refinement, chitosan has a rigid crystalline structure through inter and intramolecular hydrogen bonding.

**Solubility**
Chitosan is soluble in dilute aqueous organic or mineral acids below pH 6.5, dimethylsulfoxide, p-toulenesulfonic acid, and 10-camphorsulfonic acid.

**Properties of Chitosan**
Chitosan, a cationic polysaccharide in neutral or basic pH conditions, contains free amino groups and hence, is insoluble in water. Soluble in acidic pH with weight ranging between 3,800 and 20,000 Daltons and is 66% to 95% deacetylated. The quality and properties of chitosan products, such as purity, viscosity, deacetylation, molecular weight, and polymorphous structure, may vary widely because many factors in the manufacturing process can influence the characteristics of the final product. Particle size, density, viscosity, degree of deacetylation and molecular weight are important characteristics of pharmaceutical formulations based on chitosan. Properties such as biodegradability, low toxicity and good biocompatibility make it suitable for use in biomedical and pharmaceutical formulations. Chitosan possess mucoadhesive properties. These properties may be attributed due to strong hydrogen bonding groups like –OH, and –COOH, high molecular weight, sufficient chain flexibility, and surface energy properties favoring spreading into mucus.

**Applications of chitin/chitosan**
2.7.2 Poly (lactic-co-glycolic acid) (PLGA)
In past two decades polylactic-co-glycolic acid (PLGA) has been among the most attractive polymeric candidate used to fabricate devices for drug delivery and it has been proved for many other applications. It is a copolymer which is used in design and development of therapeutic devices and has been approved by Food and Drug Administration (FDA) owing to its biodegradability and biocompatibility.

Chemical Structure

![Chemical Structure](image)

**Figure 10.** Structure of poly (lactic-co-glycolic acid). $x =$ number of units of lactic acid; $y =$ number of units of glycolic acid

PLGA is synthesized by means of random ring-opening co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. During polymerization, successive monomeric units (of glycolic or lactic acid) are linked together in PLGA by ester linkages, thus yielding a linear, aliphatic polyester as a product.

**Solubility**
PLGA get dissolved in a wide range of common solvents such as chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate.

**Properties**
Depending on the ratio of lactide to glycolide used for the polymerization, different forms of PLGA can be obtained. These are usually identified in regard to the monomers' ratio used. All PLGA’s are amorphous rather than crystalline and show a glass transition temperature in the range of 40-60°C. Mechanical strength of the PLGA is affected by physical properties such as molecular weight and polydispersity index. PLGA has been successful as a biodegradable polymer because it undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic
acid. These two monomers under normal physiological conditions are by-products of various metabolic pathways in the body. Since the body effectively deals with the two monomers, there is minimal systemic toxicity associated with using PLGA for drug delivery or biomaterial applications. The change in PLGA properties during polymer biodegradation influences the release and degradation rates of incorporated drug molecules\textsuperscript{210}.

**Pharmacokinetic and Biodistribution of PLGA**

Biodistribution and pharmacokinetics of PLGA follows a non-linear and dose-dependent profile\textsuperscript{211}. Furthermore, previous studies suggest that both blood clearance and uptake by the mononuclear phagocyte system (MPS) may depend on dose and composition of PLGA carrier systems\textsuperscript{212}. The degradation of the PLGA carriers is quick on the initial stage (around 30%) and slows eventually to be cleared by respiration in the lung\textsuperscript{213}.

**Applications of PLGA**