Herbal medicinal products are used worldwide and have become part of clinical medicine. Today with the increasing level of consumer acceptance, safety issues related to herbal drugs continue to be ignored by the public, neglected by manufacturers as well as under-researched by the medical professionals. Users of herbal medicines tend to believe that herbs are inherently safe and thus concomitantly use self-prescribed herbal and prescribed synthetic drugs. Thus, there is a high possibility of interactions between herbal and synthetic drugs. Interactions of several commonly used herbal medicines such as Ginkgo biloba, St. John’s wort with therapeutic drugs including Warfarine, Midazolam, and Nifedipine in humans have been reported. The combined use of herbs and drugs has increased the possibility of pharmacokinetic and pharmacodynamic interactions. Herb drug interactions (HDI) involve drug metabolizing enzymes Cytochrome P450 (CYP450) and drug transporters P-glycoprotein (P-gp). As the herb drug interactions significantly alter pharmacokinetic and pharmacodynamic properties of administered drugs, the drugs interacting with herbal medicines need to be identified by appropriate in vitro and in vivo methods. In this review, we have summarized different mechanisms of herb-drug interactions mediated by CYPs and P-gp in in vitro methods to assess them.

**Keywords:** Self-prescribed, Herb-drug interactions, CYP450, P-gp, In vitro.

**Abstract**

Herbal medicinal products are used worldwide and have become part of clinical medicine. Today with the increasing level of consumer acceptance, safety issues related to herbal drugs continue to be ignored by the public, neglected by manufacturers as well as under-researched by the medical professionals. Use of herbal medicines tends to believe that herbs are inherently safe and thus concomitantly use self-prescribed herbal and prescribed synthetic drugs. Thus, there is a high possibility of interactions between herbal and synthetic drugs. Interactions of several commonly used herbal medicines such as Ginkgo biloba, St. John’s wort with therapeutic drugs including Warfarine, Midazolam, and Nifedipine in humans have been reported. The combined use of herbs and drugs has increased the possibility of pharmacokinetic and pharmacodynamic interactions. Herb drug interactions (HDI) involve drug metabolizing enzymes Cytochrome P450 (CYP450) and drug transporters P-glycoprotein (P-gp). As the herb drug interactions significantly alter pharmacokinetic and pharmacodynamic properties of administered drugs, the drugs interacting with herbal medicines need to be identified by appropriate in vitro and in vivo methods. In this review, we have summarized different mechanisms of herb-drug interactions mediated by CYPs and P-gp in in vitro methods to assess them.

**Keywords:** Self-prescribed, Herb-drug interactions, CYP450, P-gp, In vitro.

**Introduction**

Herbal medicine usage has increased throughout the world for the treatment of various disorders. General perception is that herbs are inherently safe, hence sometimes they are self-prescribed with the prescribed synthetic drugs without knowledge of the medical practitioner. When herbal medicines and conventional allopathic drugs (prescription or non-prescription medications) are used together, they can interact in our body causing changes in the way the herbs and/or the drugs work. Such changes are called ‘Herb-drug interactions’. They are harmless but sometimes really prove to be fatal [1].

The nature of herb-drug interactions is not a chemical interaction between a drug and an herb component to produce something toxic. The interaction involves an herb component causes either an increase or decrease in the amount of drug in the blood stream [1,2]. A decrease in the amount of drug occurs when herb components binds to the drug and preventing it from getting into the blood stream from the gastrointestinal tract or by stimulating the production and activity of enzyme that degrade the drug and prepare it for elimination from the body. This decrease in drug dosage could make the drug ineffective. An increase in the drug dosage could occur when an herb component aids absorption of the drug or inhibits the enzymes that break down the drug and prepare it for elimination. An increase in drug dosage could make it reach above maximum safe levels that may produce side effects or toxicity.

**Mechanism of Herb-Drug Interactions (HDIs)**

Herb-drug interactions are generally characterized by pharmacokinetic and pharmacodynamic interactions. Pharmacokinetic interactions occur when herbal formulation changes absorption, distribution, metabolism, protein binding or excretion of a drug. It involves drug metabolizing enzymes (CYP450) and drug transporters (P-gp). Some drugs are substrates for both CYP450 and P-gp thus higher probability that it will interact with herbal products. The metabolism of any drug involves two parts. Phase I reactions (biotransformation) include oxidation, hydroxylation, reduction, and hydrolysis. In these enzymatic reactions, a new functional group is introduced into the substrate molecule and an existing functional group is modified or a functional group acceptor site for phase II transfer reactions is exposed, thus making the xenobiotic more polar and therefore, more readily excreted [5,6]. This phase is carried out mainly by cytochrome P450 isoenzymes which are present in the liver these may be induced or inhibited. Phase II reactions (conjugation) are enzymatic syntheses whereby a functional group such as alcohol, phenol, or amine is masked by the addition of a new group such as acetyl, sulfate, glucuronic acid or certain amino acids which further increases the polarity of the drug or xenobiotic. Most substances undergo both phase I and phase II reactions sequentially [7, 8].

In vitro

The other major mediator of HDIs is P-glycoprotein (P-gp) transporters which are membrane bound proteins regulating the influx and efflux of drugs across the plasma membrane.

Pharmacodynamic interactions involve mutual action of herb and drug inside the body. Pharmacodynamic interactions are difficult to predict or prevent [9]. When herb and drug are taken simultaneously it may show synergistic or antagonistic effects (Fig.1).

**Methods to assess Herb-drug interaction**

To avoid or minimize toxic herb–drug interactions it is important to identify drugs that can interact with herbs using proper in vitro and in vivo models in the early stages of drug development. In vitro methods using liver or intestinal microsomes, cytosols, human hepatocytes, transfected cell lines, tumor derived cells have been widely used. In vivo methods have been reported in transgenic or humanized animals and in healthy human individuals. Each in vitro and in vivo systems has advantages and limitations (Table 1). In vitro methods are preferred over in vivo as they are less complex, easy to carry out, reliable and provides mechanistic information. It also gives information on specific CYP isoform, induction or inhibition, metabolite profile and existence of herb-drug interactions [10].

**Fig. 1:** Possible outcomes when a drug interacts with herbal medicine [17]
**IN VITRO METHODOLOGIES**

Drug metabolizing enzymes: CYP 450 assays

The CYP450 system is a family of heme based enzymes located in hepatocytes, enterocytes and smooth endoplasmic reticulum. In vitro studies with human/rat liver microsomes are conducted to evaluate ability of an herb-drug moiety to inhibit CYP1A2, CYP2D6, CYP2C8, CYP2C19 and CYP3A4. Measurement of CYP inhibition is performed by analysing inhibition of substrate metabolism. Herbs may inhibit CYP by direct competitive inhibition (reversible), non-competitive inhibition (irreversible) and mechanism-based inhibition. In reversible inhibition there is direct competition for the binding site on a CYP enzyme between the substrate and an inhibitor. Non-competitive inhibition is caused by the binding of herbal constituents containing electrophilic groups to the heme portion of CYP. The mechanism-based inhibition of CYP is due to the formation of a complex between herbal metabolite with CYP. The methods for measurement of CYP inhibition are fluorescence, luminescence, radiometric and liquid chromatography-tandem mass spectroscopy (LC-MS/MS). They are also categorized as high, medium and low throughput methods [19, 20] (Table 2).

Fluorescence and luminescence based method are high throughput methods in which pro-fluorescent or pro-luminescent substrate is metabolised to fluorescent or luminescent product. In this method CYP450 inhibition kits are used which consists of 96 well microtiter plate, NADPH (Nicotinamide adenine dinucleotide phosphate) reduced form, Tris phosphate buffer, Dibenzyl fluorescein (DBF) which acts as a fluorogenic substrate and stop reagent (acetonitrile). Drugs like Ketoconazole and quinidine are inhibitors used as a positive control. Herbal powder or individual plant material under study are extracted by using suitable extraction technique and standardized using various chromatographic techniques used like reverse phase-high performance thin layer chromatography (RP-HPTLC), Liquid chromatography-mass spectrometry (LC-MS). The assay is performed in duplicate. Control wells and blank wells are maintained. Control wells contain extracted sample, tris buffer, DBF, NADPH, water and corresponding CYP enzymes.

Blank wells contain all these except test sample. All the enzymes are freshly thaw before adding into the wells. To initiate the reaction NADPH regeneration system is added in to the wells. This system contains NADP+, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and MgCl2. Plates are incubated and reactions are stopped by adding stop reagent and evaluated for quenching and intrinsic fluorescence of the test components after 30 minutes. The values are recorded using fluorescence microplate reader (Perkin Elmer model). Rate of reaction is determined by % change in fluorescence signal. Percentage inhibition and Inhibition concentration (IC50) values are determined [25, 27].

\[
\text{Percentage inhibition} = 100 - (\text{Signal of well} - \text{Blank}) \times 100 / (\text{Solvent control} - \text{Blank})
\]

\[
\text{IC}_{50} = (\text{LP} - \text{LP}) \times (\text{HC} - \text{LC}) / (\text{HP} - \text{LP})
\]

LP = Low percentage of inhibition, HP = High percentage of inhibition, LC = Low concentration, HC = High concentration

Low throughput methods like radiometric and LC-MS/MS are also used individually or in combination. For eg. Chromatographic method combined with radiometric method for quantification of catapolo a Chinese herbal medicine. LC-MS/MS based CYP inhibition methods are also reported. Substrates are incubated with the CYP enzymes and resulting metabolites are quantified with LC-MS/MS. For eg. Evaluation of HDI during intestinal metabolism and absorption of balsalcol flavones isolated from Chinese herb [27].

**Cytochrome P450 –Carbon monoxide (CO) complex assay**

It is a simple microplate assay method. Assay is performed to determine inhibitory potential of the selected herbal formulation and it's individual bioactive compound. Carbon monoxide (CO) has a property to form complex with CYP450. The basic principle involved is mechanism based inhibition of CYP which involves following steps: 1) Preincubation of test sample with high concentration of CYP enzymes followed by dilution. 2) Incubation to measure standard enzyme activity [28].

Human/rat liver microsomes are diluted with a phospho glycerol buffer and incubated with the herbal extract under study in two different 96 well microplates. One plate is assigned as reduced P450 (P) and another is reduced P450–CO complex (PC). The reaction of herb and microsomes is initiated by addition of NADPH generating system and stopped by adding trichloro acetic acid. The PC plate is placed in the CO chamber, while the P plate is sealed with tape and placed outside the chamber. The CO chamber is designed with a plastic container and CO gas was allowed to flow into the chamber. P plate is incubated in normal

---

**Table 1: Comparison of study methods available for HDI**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro studies</td>
<td>Easy to perform, good for high throughput screenings, results are closer to human</td>
<td>Poor reproducibility of results, poor correlation to clinical situation</td>
<td>[10,11]</td>
</tr>
<tr>
<td>In vivo studies</td>
<td>Concentration and bioavailability of active components are taken into consideration</td>
<td>Results are difficult to interpret, variation in species, use of variable dosage forms. Poor statistical values.</td>
<td>[10,11]</td>
</tr>
<tr>
<td>Case report studies</td>
<td>Providing information on HDI.</td>
<td>Expensive, genetic variation in enzyme activity.</td>
<td>[9,10]</td>
</tr>
<tr>
<td>Human studies</td>
<td>Extrapolative data on interactions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Methods for inhibition of CYP**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Mechanism</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence (high throughput)</td>
<td>Pro-fluorescent substrate metabolized to fluorescent product.</td>
<td>Fast, sensitive, cost effective, measures enzyme activity.</td>
<td>Recombinant enzymes needed as probes.</td>
<td>[19,20,34]</td>
</tr>
<tr>
<td>Luminescence (high throughput)</td>
<td>Pro-luminescent substrate metabolized to luminescent product.</td>
<td>Very sensitive.</td>
<td>Work-up reagent required. Recombinant enzymes needed.</td>
<td>[25,27]</td>
</tr>
<tr>
<td>Radiometric (low throughput)</td>
<td>Release of radiolabel on metabolism of substrate</td>
<td>Fast and accurate.</td>
<td>Large amount of radiolabelled substance used.</td>
<td>[19,20]</td>
</tr>
<tr>
<td>LC_MS/MS (Medium throughput)</td>
<td>Standard method for all interaction assessment.</td>
<td>Sensitive and specific.</td>
<td>Expensive</td>
<td>[28,30]</td>
</tr>
<tr>
<td>CYP450–CO complex assay</td>
<td>CYP450 microsomes quantitated using characteristic absorbance peak at 450nm</td>
<td>Simple, less laborious, reliable, reproducible</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ref.**

[6]
[9,10]
[10,11]
condition, while PC plate is incubated in the CO chamber for 15 min. All the samples are reduced by adding freshly prepared sodium hydrosulphite solution (SHS). At this stage, the yellow colour of the PC sample was visible whereas the P sample remained colourless or turned pale pink. Ketonozolane is an inhibitor used as positive control. The difference in absorbance of the samples at 450nm and 490nm was measured with a microplate reader. Since, reduced P450-CO complex produces a characteristic absorption peak at 450nm than reduced P450 [28,30]. Concentration of CYPs was calculated using the formula: 

\[ [\text{CYP450}] \text{(mM)} = \frac{(\Delta \text{APC} - \Delta \text{AP})}{91} \]

Where \( \Delta \text{APC} \) = absorbance difference of the PC sample, and \( \Delta \text{AP} \) = absorbance difference of the P sample, 91 is the extinction coefficient.

Percentage inhibition is determined.

Percent inhibition = \( \frac{\text{Blank} - \text{Test}}{\text{Blank}} \times 100 \)

**Drug Transporters: P-glycoprotein (P-gp) assay**

P-glycoprotein (P-gp) transporters are membrane bound proteins regulating the influx and efflux of drugs across the plasma membrane. It is found in cell membranes which affects serum drug levels by blocking or facilitating entry into cells. Also found in tissues intestine, liver and kidney. It is used widely because of simple procedure, low sample requirement, low cost, high speed and can determine IC50 value over a concentration range of >1000 [31,33]. Assay is performed to determine functional activity of P-gp in cells like colon carcinoma cells (CaCo) or hepatic cells by measuring Rhodamine 123 (Rh123) retention/efflux. Rh123 is a cationic, fluorescent dye taken up by functional activity of P-gp in cells like colon carcinoma cells (CaCo) or hepatic cells by measuring Rhodamine 123 (Rh123) retention/efflux. Rh123 is a cationic, fluorescent dye taken up by functional activity of P-gp in cells like colon carcinoma cells (CaCo) or hepatic cells by measuring Rhodamine 123 (Rh123) retention/efflux. Rh123 is a cationic, fluorescent dye taken up by functional activity of P-gp in cells like colon carcinoma cells (CaCo) or hepatic cells by measuring Rhodamine 123 (Rh123) retention/efflux. Rh123 is a cationic, fluorescent dye taken up by

**REFERENCES**


