Chapter 1: Origin and Importance of the Research Plan

1.1. Introduction

Nature has provided seemingly endless source of medicine and therapeutic benefits of many natural products remains unchallenged [1]. Herbs in their crude form have long been and continue to be the basis of many traditional medicines while herbal products including herbal medicines, nutraceuticals and dietary supplements have increasingly become popular as alternative medicines worldwide. Herbal medicines are, more than ever, receiving attention, both from the public and healthcare professionals alike, with many countries now undertaking registration schemes for traditional medicines. However, healthcare professionals still freely admit their lack of knowledge in this area, and surveys suggest that patients often rely on friends and family for advice about herbal medicines. Never has there been a more appropriate time to advise healthcare professionals so that they can provide balanced, helpful advice to patients wishing to take herbal medicines with their "conventional" treatments. The global increase in popularity of alternative medicines is therefore quite evident and this has raised renewed concerns regarding herb drug interaction or regarding interactions between individual components of any herbal chemotype and any concomitantly administered allopathic drug [2-4]. The safety of herbal products continues to be a matter of concern, even though toxic herbs have been eliminated from products manufactured in developed countries. In developing countries it still remains unorganized and unregulated. Herbal medicines may contain ingredients in addition to active components that may have a profound effect on the disposition of drugs.

The potential for herb–drug interactions has been highlighted by the recognition that St. John's wort (Hypericum perforatum) may interact with certain prescription medicines, including HIV protease inhibitors, oral contraceptives, selective serotonin reuptake inhibitors (SSRIs), theophylline, cyclosporin and warfarin [5-10].

Disquiet over the safety of herbs used in herbal products, whether licensed as medicines or sold as unlicensed products, is reflected by medicines regulatory authorities world–wide, highlighting the need for health-care professionals to be aware of such problems. As the incidence and severity of herb drug pharmacokinetic interactions increase due to worldwide rise in the use of herbal preparations, more preclinical or clinical data regarding herb drug pharmacokinetic interactions are needed to make informed decisions regarding patient safety.
Keeping in mind the above facts, the following aspects are of utmost importance and are highly relevant in national and international scenario:

1. Most important implication of the global increase in natural product usage is the probability of an increase in herb–drug interactions.

2. Herb–drug interactions may be pharmacokinetic or pharmacodynamic in nature. Pharmacokinetic interactions are more frequently reported, but may be more difficult to be characterized mechanistically as they often occur through multiple mechanisms.

3. Herb–drug pharmacokinetic interactions become clinically significant when considerable changes occur in the pharmacokinetic parameters (Cmax, Tmax and AUC) of the co-administered drug in humans, especially when the drug has a narrow therapeutic index (e.g., digoxin, warfarin and phenytoin) and, therefore, is prone to result in toxic effects.

4. Herbs may change the pharmacokinetics of co-administered drugs by altering the drug’s absorption, distribution, metabolism and excretion through various mechanisms.

5. Oral drug absorption is modulated by inhibition or induction of either efflux or uptake drug transporters, which may result in loss of therapeutic effect or occurrence of toxic effects, while controlled inhibition of drug efflux may be used as an oral drug absorption enhancement approach.

6. Many potentially dangerous herb–drug interactions are due to modulation of Phase I and II metabolizing enzymes in the gastrointestinal epithelium and the liver.

7. The upregulation of CYP3A4 enzyme and P-glycoprotein expression by St John’s Wort through pregnane X receptor and the mechanism-based inhibition of intestinal CYP3A4 by grapefruit juice suggest that other plant products that occupy the same pathways may cause similar herb–drug interactions.

8. Polymorphism in genotypes of metabolic enzymes and drug transporters found in certain ethnic groups may influence herb–drug interactions mediated through these pathways.

9. Altered gastrointestinal motility, complex formation, displacement from plasma proteins, change in pH of physiological fluids, or modulation of drug transporters in
hepatocytes and renal tubules following consumption of herbal products can have a marked impact on the therapeutic outcome of treatment with conventional drugs.

10. Future research is needed to identify potentially harmful herb-drug pharmacokinetic interactions that are yet unknown and to provide information regarding clinical significance in long-term use of most herbal products in conjunction with other medicines and/or other herbal medicines.

Many of these herbal preparations contain flavonoids as the major constituents that play a vital role in pharmacokinetic interactions leading to changes in drug efficacy or toxicity. Flavonoids are part of a number of dietary supplements, nutraceuticals and herbal medicines for example Red Clover, Soybean, St John’s wort, Grape fruit juice and Ginkgo Biloba (Glycine max L.). In recent years, there has been a resurgence of scientific interest in flavonoids with more than 55000 publications containing “flavonoids” as a key word upon making a search in Medline/NCBI, National Library of Medicine, USA (PubMed). This is due to the association of these compounds with a wide range of health promoting effects and general belief among the public that herbal and dietary preparations are “good for humans” as they are “all natural”. Numerous studies have indicated that flavonoids have anti-oxidant, anti-carcinogenic, anti-viral, anti-inflammatory and anti-estrogenic or estrogenic activities. Dietary intake of flavonoids has been linked with reduced risk of cancer, osteoporosis, cardiovascular diseases, and other age-related degenerative diseases [11-16]. Due to their wide variety of health-beneficial activities, numerous herbal preparations containing either flavonoid glycosides or aglycones, are now being marketed in various formulations as dietary and herbal supplements, for example, red clover (Trifolium pratense), grape fruit juice, soy products and milk thistle (Silybum marianus) etc. Unfortunately, herbal products and/or dietary supplements are not tested with the stringent scientific rigor required by conventional medicines and do not undergo regulation and approval process by the regulatory bodies for marketing permission. It has been estimated that herbal products are ingested by more than 10% or more of the general population and 30-70% of individuals with specific disease states [17]. Thus, the dietary consumption of large doses of flavonoids is frequent, increasing the possibility of flavonoid-mediated pharmacokinetic interactions with conventional medication. A drug interaction is defined as any modification caused by another exogenous chemical (drug, herb, or food) in the diagnostic, therapeutic, or other action of a drug in or on the body.
Herb-drug interaction can occur through several different mechanisms. Herb-drug interactions can be pharmacodynamic or pharmacokinetic in nature (Figure-1.1. and 1.2).

Figure-1.1. Possible mechanisms for drug interactions with combined herbal medicines. As for drug–drug interactions, both pharmacokinetic and pharmacodynamic components may play important roles in herbal interactions with prescribed drugs.

PgP = P glycoprotein; MRP = multidrug resistance associated protein; UGT = uridine diphosphate-glucuronosyltransferase.
Figure-1.2. Possible clinical outcomes when a drug interacts with combined herbal medicines.

AUC = area under the curve
Pharmacodynamic herb-drug interactions occur when herbal and/or dietary components and drugs share a common pharmacologic mechanism of action or when a pharmacokinetic interaction leads to an altered pharmacologic profile. The pharmacokinetic herb-drug interaction may take place in any and/or multiple place(s) in the body e.g. in gastrointestinal tract mediating through absorption (e.g. modulation of efflux and uptake transporters, complex formation, GI motility and pH), in liver mediating through metabolism (e.g. induction or inhibition of metabolizing enzymes/transporters) and in kidney mediating through renal clearance mechanisms. These herb-drug pharmacokinetic interactions mostly occur due to modulatory effect of herbal components on drug metabolizing enzymes and transporters. Till date several significant herb-drug interactions have been reported in the literature (Table 1.1.) [18-37].
### Table-1.1. Drugs that interact with herbal medicines in human

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Interacting herb</th>
<th>Constituents</th>
<th>Interaction outcome</th>
<th>Possible mode of action</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alprazolam</td>
<td>SJW</td>
<td>Rutin, isoquercetin, quercetin, hyperisin</td>
<td>▼ AUC by 41%, t1/2 by 24%, and Cmax by 15%</td>
<td>Induction of CYP3A4</td>
<td>22</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>SJW</td>
<td>Rutin, hyperoside, isoquercetin, quercetin, hyperisin</td>
<td>Nifedipine: ▼ AUC by 44.9%, Cmax by 38.5%</td>
<td>Induction of CYP3A4</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dehydronifedipine: ▲ AUC by 25.7%, Cmax by 55.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>SJW</td>
<td>Rutin, hyperoside, isoquercetin, quercetin, hyperisin</td>
<td>▼ 13–15% in norethindrone and ethinyl estradiol levels</td>
<td>Enzyme induction</td>
<td>24</td>
</tr>
<tr>
<td>Digoxin</td>
<td>SJW</td>
<td>Rutin, hyperoside, isoquercetin, quercetin, hyperisin</td>
<td>▼ AUC by 25%, Cmax by 33%</td>
<td>P-gp induction</td>
<td>25</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>SJW</td>
<td>Rutin, hyperoside, isoquercetin, quercetin, hyperisin</td>
<td>▼ Cmax &amp; AUC of simvastatin hydroxy acid</td>
<td>Induction of enzyme and P-gp</td>
<td>26</td>
</tr>
<tr>
<td>Tinidactan</td>
<td>SJW</td>
<td>Rutin, hyperoside, isoquercetin, quercetin, hyperisin</td>
<td>▼ SN-38 by 42%</td>
<td>Modulation of P-gp</td>
<td>27</td>
</tr>
<tr>
<td>Indinavir</td>
<td>Milk Thistle</td>
<td>Silymarin (silybin, silichristin, silydianin)</td>
<td>▼ AUC by 9%</td>
<td>Modulation of CYP3A and P-gp</td>
<td>28</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Garlic</td>
<td>Allin, allicin</td>
<td>Increased sulfation</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Propranolol</td>
<td>Piperine</td>
<td>-</td>
<td>▲ Cmax and AUC</td>
<td>CYP1A2 inhibition</td>
<td>30</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>Garlic</td>
<td>Allin, allicin</td>
<td>▼ AUC by 51%, Cmax by 54%</td>
<td>Induction of CYP3A4 and P-gp</td>
<td>31</td>
</tr>
<tr>
<td>Talinolol</td>
<td>Ginkgo biloba</td>
<td>Gingkolide A, B, C, J, M</td>
<td>▲ AUC and Cmax</td>
<td>Inhibition of P-gp</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>quercetin, kaempferol</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Midazolam</td>
<td>Ginkgo biloba</td>
<td>Gingkolide A, B, C, J, M</td>
<td>▼ Cmax and AUC</td>
<td>Induction of CYP3A4</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>quercetin, kaempferol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>Grapefruit</td>
<td>Flavonoids and furanocoumarins</td>
<td>▼ AUC</td>
<td>Inhibition of OATP1A2</td>
<td>34</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Grapefruit</td>
<td>Flavonoids and furanocoumarins</td>
<td>▲ AUC</td>
<td>Inhibition of CYP3A2</td>
<td>35</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Diosmin</td>
<td>-</td>
<td>▲ AUC and Cmax</td>
<td>Inhibition of CYP2C9</td>
<td>36</td>
</tr>
<tr>
<td>Buspirone</td>
<td>Green tea</td>
<td>Polyphenols (catechins and flavonoids)</td>
<td>▲ AUC</td>
<td>Inhibition of CYP3A4</td>
<td>37</td>
</tr>
</tbody>
</table>

AUC: area under the plasma concentration-time curve; Cmax: Maximum Plasma concentration.
SJW: St. John's Wort; t1/2: elimination half life; ▼ decrease; ▲ increase.
In general, adverse reactions are generally associated with drugs/herbs able to inhibit or induce drug metabolizing enzymes or transporters [38-40]. These mainly include Cytochrome P450s (CYPs), uridine diphosphate-glucuronosyltransferase (UGTs), efflux (P-Glycoprotein, P-gp; MRP-2; BCRP) and uptake transporters (organic anion-transporting polypeptides, OATPs). Cytochrome P450 monooxygenases are heme containing mixed function oxidases playing a key role in metabolism of hydrophobic endogenic substrates (sterols, prostaglandins, fatty acids) and xenobiotics (e.g. drugs, carcinogens, food components, pollutants). The Cytochrome P450 enzyme system consists of several isoforms like CYP1A2, CYP2D6, CYP2B6, CYP2C9, CYP2C19 and CYP3A4 etc. and each of these can metabolize multiple substrates. Flavonoids may interact with other compounds by inducing biosynthesis of CYPs or modulation of activity of these enzymes. These are also found to be substrate of several CYPs and therefore competitive inhibition may be the cause of interaction. Efflux transporters (P-gp, MRP2 and BCRP) located within the polarized apical membrane of the intestine, liver and kidney mediating the efflux of xenobiotics and toxins into the intestinal lumen, bile and urine [41]. MRPs and P-gp are found to be expressed with CYP3A4, glutathione-S-transferases, UDPglucuronosyltransferases [42, 43], which may play the synergistic function in regulating the bioavailability of many orally ingested compounds. Therefore, the modulation of these transporters may have significant pharmacokinetic consequences. On the other hand, the interaction of flavonoids with these enzymes and transporters may be exploited as a way to improve pharmacokinetic properties of the co-administered drug. Since the pharmacokinetics and/or pharmacodynamics of the drug may be altered by combination with herbs/flavonoids, such interactions can have serious effects to the patients on co-administration; may lead to adverse side effects or even life-threatening conditions due to increased drug levels above the toxicity threshold or can be ineffective due to decreased drug levels below the effective concentration.

Despite of the clinical significance of drug interactions with flavonoids present in different herbal and dietary preparations there are only few scientific reports describing the quantification of these compounds in biological matrices, and also their potential involvement in drug interactions is largely unknown. Therefore, it is imperative to gain thorough knowledge on absorption, metabolism and pharmacokinetics of flavonoids; & their interaction with clinically used drugs. In the current work, we propose to study the absorption,
metabolism and pharmacokinetics of commonly consumed flavonoids (biochanin A, formononetin and kaempferol); and their effect on pharmacokinetics of clinically used drugs (tamoxifen, raloxifene and centchroman) requiring chronic administration.

Many investigators have shown that a class of plant-derived substances had estrogenic activities, they are so called “phytoestrogens”. These include the flavonoids family comprising isoflavones (e.g. biochanin A, formononetin, isoformononetin, glycitein, daidzein, and genistein) and flavonols (e.g. kaempferol, myricetin and quercetin) etc. There has been considerable scientific interest in the role of these phytoestrogens in numerous aspects of human health. Of particular focus have been the diseases and/or conditions—including breast cancer, prostate cancer, other types of cancer, cardiovascular disease and osteoporosis, as well as alleviation of menopausal symptoms. These phytoestrogens have the beneficial effects of estrogen without the negatives, especially in tissues such as the endometrium and breast [44]. Biochanin A (BCA), formononetin (FMN) and kaempferol (KMF) have been shown to reduce the occurrence of osteoporosis in various experimental models [45-48].

BCA and FMN are principal phytoestrogens in red clover preparations, such as Promensil (Novogen, Inc., Samford, CT, USA)) that are marketed and sold as dietary supplements for relieving postmenopausal symptoms such as hot flashes, bone loss, breast cancer and for maintaining men’s prostate health. In various in vitro and in-vivo studies, BCA and FMN shown to be potent inhibitor of the efflux transporters viz. P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), which are important molecular mechanisms for both multidrug resistance and drug disposition [49-52].

KMF is one of the most commonly found dietary flavonols. It is found in grapefruit, Ginkgo Biloba L. (Ginkgoaceae), broccoli and other plant sources, and accounts for approximately 25–33% of the mean dietary flavonol intake in the USA [53-55]. KMF has been suggested to play a role in preventing postmenopausal conditions, namely cardiovascular disease, hot flushes and osteoporosis [56]. KMF can also induce growth arrest and apoptosis in colorectal, lung and prostate cancers through inhibiting DNA synthesis, inducing nuclear DNA degradation, and inhibiting kinase activities [57-59]. KMF have also been reported to inhibit the enzymatic activities of some phases I and II drug metabolizing enzymes including
CYP1A1, CYP1B1, CYP1A2, CYP3A4, and sulfotransferase 1E1 (SULT1E1) and SULT1A2 [60, 61].

The diverse biological activity of phytoestrogens is due in part to their ability to act estrogendically as estrogen agonists and antiestrogendically as antagonists. As estrogen agonists, phytoestrogens mimic endogenous estrogens and cause estrogenic effects. As estrogen antagonists, they may block or alter estrogen receptors (ER) and prevent estrogenic activity, causing antiestrogenic effects [62]. As estrogen agonists and antagonists, phytoestrogens can also be classified as natural selective estrogen receptor modulators (SERMs) [62].

SERMs are non-steroidal chemicals with a similar structure to 17 β-estradiol (E2) and an affinity toward estrogen receptors [63]. They are unique in that they can function as agonists or antagonists depending on the tissue, ER and concentration of circulating endogenous estrogens [64]. Tamoxifen and raloxifene are well-known synthetic SERMs. Tamoxifen, the prototypical SERM, has been used in clinical practice for breast cancer patients because it acts as an estrogen antagonist in breast tissue, slowing cancer cell proliferation and an estrogen agonist in bone tissue and in the cardiovascular system to prevent osteoporosis and heart disease. However, tamoxifen has shown estrogenic activity in the uterus and therefore may increase the risk of endometrial cancer [65, 66]. As a result of tamoxifen’s potential side effects, several second generation SERMs have been developed to reduce potential toxicities. Oral tamoxifen undergoes extensive hepatic metabolism and the subsequent biliary excretion of its metabolites. In humans, the main pathway in tamoxifen biotransformation proceeds via the N-demethylation catalyzed mostly by CYP3A4 enzymes [67, 68]. Among the serum metabolites of tamoxifen, 4-hydroxytamoxifen has received particular attention since it has higher in-vitro affinity towards the estrogen receptor than the parent drug. It has been reported to be about 100 times more potent as an estrogen antagonist than tamoxifen and its plasma and tumor concentrations found only about 2% of those of the parent compound [69, 70]. 4-hydroxytamoxifen, is produced in humans by CYP2D6, CYP2C9, CYP2E1 and CYP3A4 [67, 68, 71]. Tamoxifen and its metabolite 4-hydroxytamoxifen are substrates of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance-associated protein (MRP) 2 [72, 73]. Since, the tamoxifen is a substrate of P-gp and undergo extensive CYP450 mediated metabolism makes it vulnerable for interactions with commonly consumed phytoestrogens.
Raloxifene, a non-hormonal agent, is a second generation SERM that binds to the estrogen receptor and results in estrogen agonist effects on bone. It is used clinically for the treatment and prevention of osteoporosis and breast cancer in postmenopausal women. Raloxifene has shown to be as effective as tamoxifen in reducing breast cancer, with a reduced risk of endometrial cancer and blood clots [74, 75]. Despite its rapid absorption in humans following oral administration, raloxifene bioavailability is significantly lower (2%) [76]. The low bioavailability of raloxifene is mainly due to its extensive biotransformation, but it does not appear to be metabolized by the cytochrome P450 pathway [77]. Raloxifene mainly undergoes extensive Phase II mediated metabolism. Therefore, there could be the potential metabolic interaction between raloxifene and drugs/herbal constituents eliminated by the Phase II metabolism. BCA, FMN and KMF are known to undergo extensive Phase II (mainly glucuronidation and sulfation) metabolism.

Centchroman (Ormeloxifene) is a nonsteroidal, selective estrogen receptor modulator and a once-a-week oral contraceptive agent developed by Central Drug Research Institute, Lucknow, India [78, 79]. Centchroman has been reported to exhibit partial to complete remission of lesions in 40.5% breast cancer patients due to its potent antiestrogenic and weak estrogenic activities [80, 81]. It also possesses desirable bone protective [80, 82, 83] and lipid lowering activities [84]. Centchroman is known to be extensively metabolized by liver [85]. Further, long term administration of centchroman by women makes it vulnerable for interactions with widely consumed herbal and dietary supplements.

Most of the climacteric and postmenopausal women appears to have vasomotor symptoms and high risk of osteoporosis and cardiovascular disease. Exogenous estrogens (hormone replacement therapy, HRT) given to these women have known to reduce the risk of these symptoms and diseases. However, women are reluctant to use HRT because of undesirable side-effects (such as irregular bleeding) and safety (especially risk of breast cancer) [62]. SERMs have been shown to be a possible new alternative for postmenopausal therapy [86]. Therefore, postmenopausal women are increasing their consumption of phytoestrogens (natural SERMs) containing products. Dietary supplements and herbal preparations containing phytoestrogens are heavily marketed for the treatment of osteoporosis, cancer and for the alleviation of menopausal symptoms. The combination of phytoestrogens (natural SERMs) and synthetic SERMs may be desirable because they may have synergistic effect
against osteoporosis and breast cancer [86, 87]. However, studies concerning whether phytoestrogens would favorably or adversely affect the pharmacokinetics of synthetic SERMs are largely unknown.

1.2. Aims and Objectives of the Study:

Primary Objective:

In the current work, we propose to study the absorption, metabolism and pharmacokinetics of commonly consumed flavonoids (biochanin A and formononetin) and their effect on pharmacokinetics of clinically used drugs (tamoxifen, raloxifene and centchroman) requiring chronic administration.

Secondary Objective:

- Standardization of Parallel artificial membrane permeability assay (PAMPA)
- Standardization of In-situ Permeability Model
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1.3. References:


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