2. Literature Review - Gaps - Aim and Objectives

2.1. Literature Review

More than 60% of the world's population use traditional medicines. Remarkably, a major portion of their consumers is from developed countries, however, India remains one of the key consumers for herbal or traditional medicines. Around 2/3rd of the rural Indian population practices the traditional medicinal system for the treatment of various diseases and > 20,000 plants have been documented with remedial potential [1,2]. There is an increased interest in the general public for the therapeutic efficacy of traditional or herbal medicines. This mindfulness is related to an understanding that herbal drugs improve immunity, the body's restorative power and natural resistance to various ailments. Generally, these traditional or herbal medicines are believed to be 'safe' and deprived of side effects as they are mostly of botanical origin and are often constituents of food or dietary supplements. Moreover, they are co-administered with therapeutic drugs from the allopathic system of medicine. The combined use of traditional or herbal medicines with allopathic drugs may decrease, increase or mimic the effects of either constituent, which may result in herb-drug interactions. Several frequently used traditional or herbal medicines have been reported to modulate the pharmacokinetics of various prescribed medicines, leading to altered pharmacokinetic properties. The renowned clinical case is St. John's Wort that led to a decrease in the area under the curve (AUC) of a variety of clinical drugs, including but not limited to cyclosporine and warfarin [2-10]. The results owing to some of these interactions could be lethal, such as St John's Wort reducing cyclosporine's systemic concentration and that may result in tissue rejection in patients who have undergone an organ transplant. Therefore, co-administration of herbs with therapeutic drugs with a narrow therapeutic range is on the risk, (e.g., warfarin and theophylline) and for high-risk groups, such as the geriatric population or patients with renal or hepatic disorders. Figure 2.1 indicates some of the reported herb-drug interactions for commonly used herbs with different classes of modern therapeutic drugs. For evaluation of similarly acting drugs, these are categorized using their pharmacologic drug class, e.g., rosiglitazone, glibenclamide, metformin, and chlorpropamide are grouped as oral hypoglycemics.

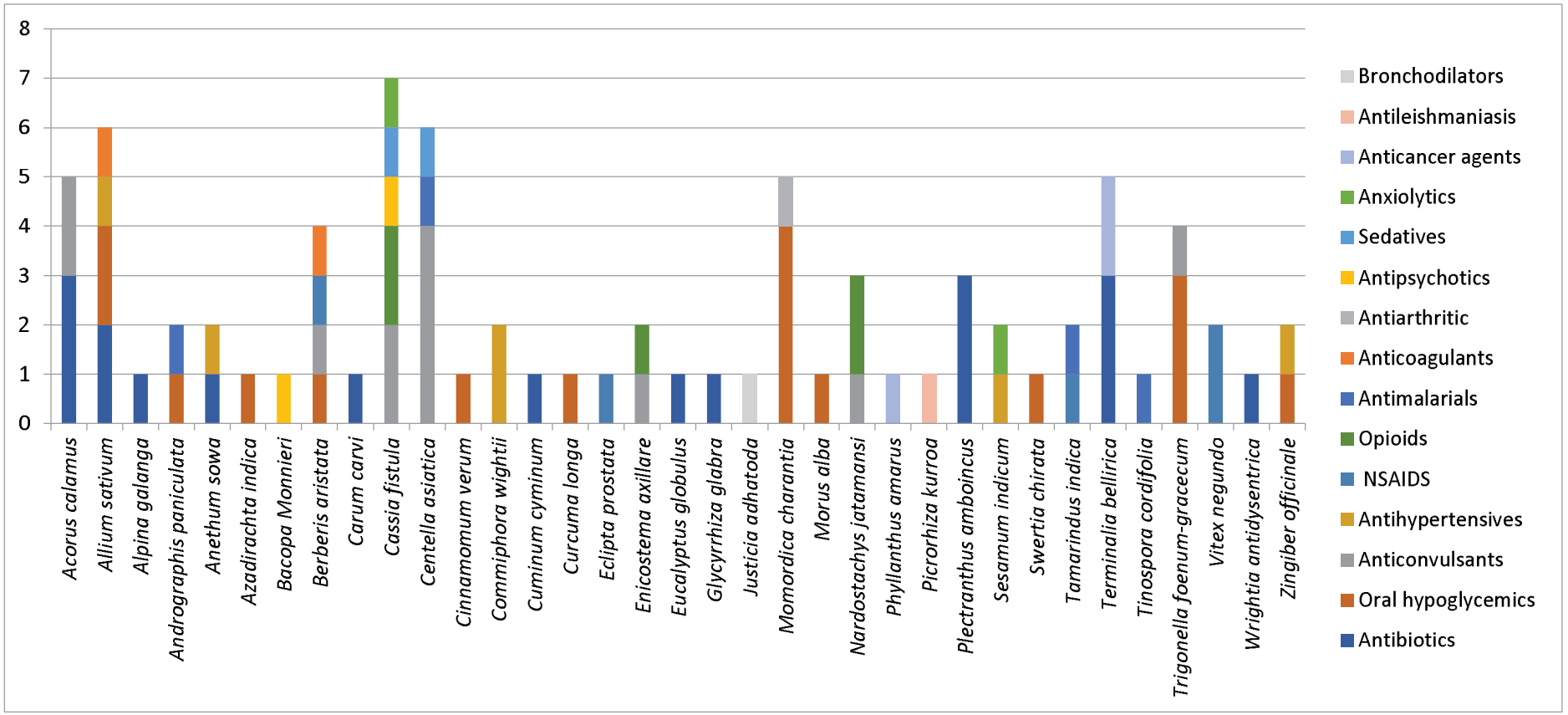

Figure 2.1. Known interactions of commonly used herbs with drugs from different therapeutic classes. {Within a class of drugs, each interaction with a member drug is counted as one interaction and accordingly total number of different drugs within each category of drugs with potential for interaction are plotted on Y-axis against each herb on X-axis}.

2.2. Mechanism of Herb-Drug Interactions

In addition to drug-drug and food-drug interactions, the superimposing substrate specificity in the various metabolism pathways is the leading source for herb-drug interactions. The diverse chemical moieties can interact with receptor sites and modify physiological response that can describe pharmacodynamic drug interactions while pharmacokinetic interactions happen as a result of altered absorption, distribution, metabolic and excretory pathways [11]. The major fundamental mechanism of pharmacokinetic herb-drug interactions is either the inhibition or induction of intestinal and/or liver DME (drug metabolic enzymes), predominantly the cytochrome P450 (CYP) enzymes. Moreover, the comparable effect on drug transporters including uptake and efflux proteins particularly the P-glycoproteins (P-gp) in the intestines and the blood-brain barrier is responsible in most of the cases [12,13]. The intestinal and/or liver activity of CYP and transporters often impact oral bioavailability, therefore modulating the DME and or transporter activity with co-administered herbal products has been reported to impact significantly the systemic concentration of victim drugs [14].

The interaction of herbal products with hepatic enzymes can also result in pharmacodynamic (PD) effects [15-18]. Specific liver injury (hepatitis) by natural products comprises elevation in transaminase, acute & chronic hepatitis [19-21], liver failure, veno-occlusive disorders, liver cirrhosis, fibrosis, cholestasis, zonal or diffusive hepatic necrosis, and steatosis. The mechanism behind liver injury may comprise CYP bioactivation, mitochondrial injury, oxidative stress and apoptosis [22-28].

2.2.1. CYPs and their role in Herb-Drug Interactions

The CYP family of DME is generally involved in reductive, oxidative and peroxidative, biotransformation of endogenous components, such as bile acids, hormones, and fatty acids and xenobiotics. CYP is divided into families and subfamilies and different families demonstrate a high degree of substrate specificity. CYP (1, 2, and 3) families are mainly involved in drug metabolism. The most significant CYP subfamilies responsible for xenobiotic metabolism in human beings are 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5 [29-32].

CYP1A1/A2 are the key enzymes of the human CYP1A subfamily. CYP1A1 is mostly expressed in extra-hepatic tissues (intestine, kidney and lung) whereas CYP1A2 is found in the liver and constitutes ~ 15% of total liver CYP. Most enzymes of the CYP2B subfamily play a less significant role in drug metabolism except CYP2B6 [33,34]. The second most abundant subfamily 2C after 3A (amounting to ~20% of the total CYP present in the liver of human beings). It includes three members: 2C8, 2C9, and 2C19, and these are also involved in the

biotransformation of endogenous components including retinoic acid and retinol. Sporadic clinically pertinent drugs including paracetamol, chlorzoxazone, and enflurane are metabolized by CYP2E1 (most active from the 2E subfamily). CYP3A subfamily comprises over 40% of the total CYP in the body of human beings (although the inter-individual variabilities are observed) with CYP3A4 with the maximum abundance in the liver and intestines and responsible for the metabolism of about 50% of drugs in use today. The substrates and inhibitors specificity and selectivity for these DMEs are particularly useful in understanding pharmacokinetic and toxicological/toxicokinetic studies [35-38].

Induction means the increase in enzyme activity (mostly intestinal and liver) as a result of amplified mRNA transcription leading to increased protein levels, more than normal physiologic conditions. If this occurs (induction), there is an equivalent increase in the rate of drug biotransformation affecting both bioavailability and the systemic disposition of the victim drug. In the dosage design of oral medications (formulation strategies), allowance is often made for the first-pass metabolism to attain predictable bioavailability. A disturbance in this equilibrium may result in significant changes in the systemic concentration of the victim drugs. Certain herbal products have been reported to induce DME and transporters. Concomitant administration of DME-inducing herbal products and victim drugs can therefore result in decreased (sub-therapeutic) systemic levels of the victim drugs leading to therapeutic failure as a probable clinical consequence.

In addition to enzyme induction, herbal products can also inhibit enzyme activities. The inhibition of DME is usually competitive with rapid and inhibitor concentration-dependent effects. Most competitive inhibitors are also substrates of the DMEs. This phenomenon significantly alters pharmacokinetic profiles of co-administered victim drugs. Inhibition of the anticipated intestinal and hepatic metabolism remarkably results in high systemic levels of co-administered victim drugs. Lethal manifestation could be the eventual result of this observation. These effects will be of particular importance with the drugs having a narrow therapeutic window [39, 40].

Many herbs are described to exhibit interactions with the major CYP enzymes. Both induction and inhibition of CYP450 activity/expression are described. Figure 2.2 is a compilation of data from the reported studies for inhibition or activation of various families of CYP enzymes by herbs. CYP1A1 and CYP3A4 are on top of the list, followed by CYP1A2 and CYP2C9. The herbs, *Andrographis paniculata, Salacia reticulata, Allium sativum,* are identified to induce CYP1A1 although herbs*, Phyllanthus amarus*, *Berberis aristata, Curcuma longa, Carum carvi,* are known for inhibition of CYP1A1. Similarly, herbs like *Glycyrrhiza glabra and*

Commiphora wightii induce CYP3A4 and herbs, *Myristica fragrans*, *Aesculus indica, Acorus calamus, Centella asiatica, Andrographis paniculata* and *Curcuma longa* are reported to inhibit the CYP3A4 enzyme. Moreover, most herbs interacted with more than one CYP enzyme. *Andrographis paniculata* exhibited interactions with eight CYP enzymes (CYP2C9, CYP2C11, CYP2D6, CYP3A4, CYP1A1, CYP1A2, CYP2C6 and CYP3A1/2). The level of interaction also depends on the dosage of the administered natural product. For example, in rats, a high dose (500 mg/kg, *i.p.*) of garlic oil decreased hepatic CYP activity, while a low-dose (50 mg/kg, *i.p.*) of garlic oil increased CYP450 activity.

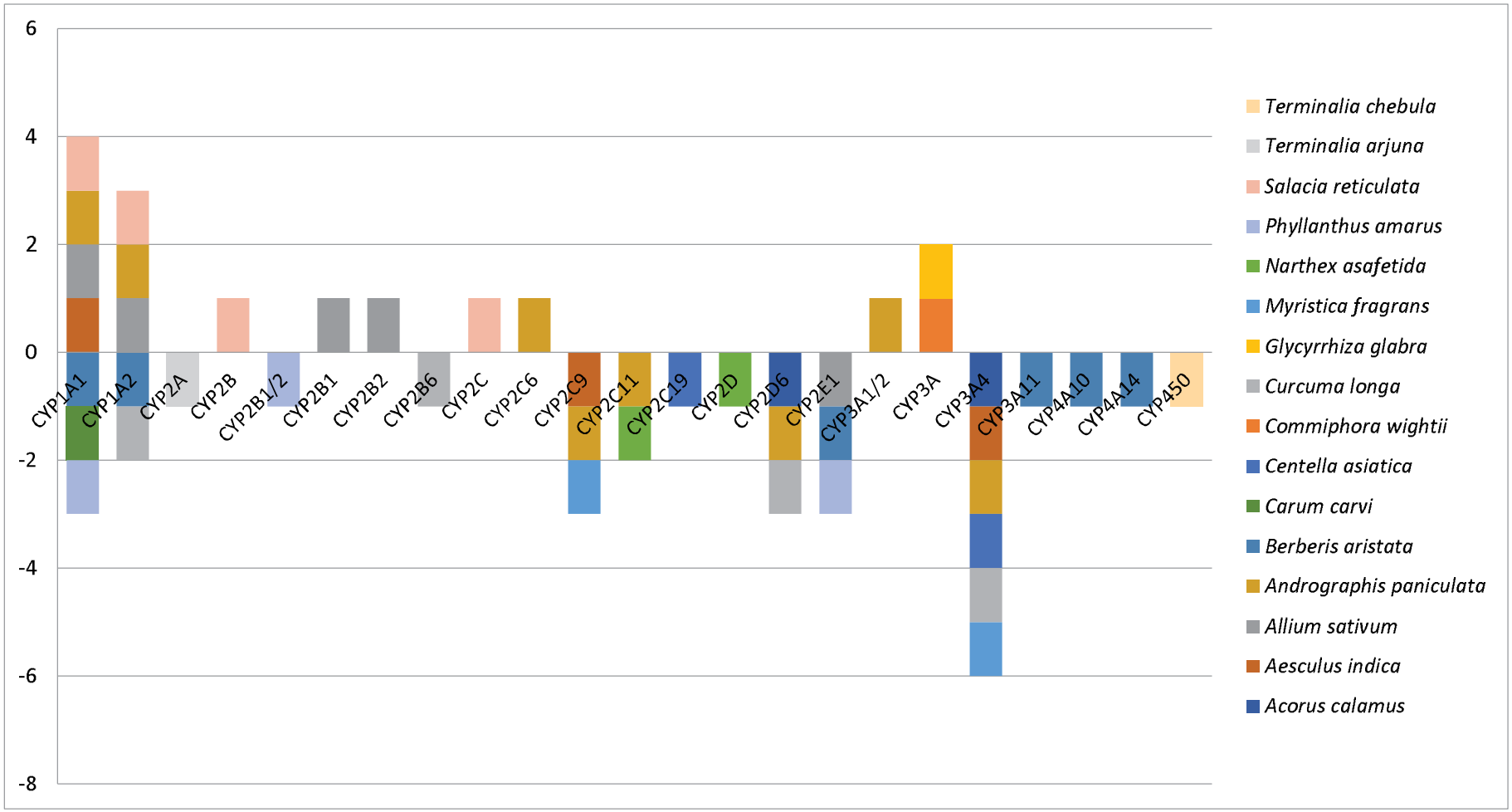

Figure 2.2. Known interactions (both activation and inhibition) of commonly used herbs with different CYP enzymes. {Activation is depicted by the positive Y-axis, while inhibition is depicted by the negative Y-axis. Different sub-families of CYP are plotted on X-axis}.

The number of CYPs mediated interactions that are reported while using herbal medicines is depicted in Table 2.1. As evident, CYP1A1 and CYP3A4 are reported to have a maximum number of interactions followed by CYP1A2 and CYP2C9 [41-55]. Some of the prominent interaction includes reduction of bioavailability of CYP3A4 substrates with *Commiphora wightii* and *Glycyrrhiza glabra* due to the induction of the pregnane X receptor (PXR) and consequent induction of CYP3A [46,49,51], decrease in protein expression of intestinal P-gp and CYP3A4 in rats by treatment with curcumin for 4 days (dose: 60 mg/kg/day) [53] etc. Other interactions are reported for herb *Myristica fragrans*, *Aesculus indica, Curcuma longa, Andrographis paniculata and Centella asiatica,* that have terpenoids as major natural products [47,48,53,56].

It is important to note here that most of the reported studies were made using extracts of herbs and not using pure natural products. An extract may have numerous components with varying compositions, efforts on the evaluation of specific constituents may deliver a better understanding to which constituent/natural products is exactly accountable for the interaction. In very few studies, individual natural products have been explored. For e.g. Escin (major constituent in *Aesculus indica),* showed mixed effects on CYP enzymes. Single and multiple doses of escin inhibited CYP2C9 and CYP3A4, induced CYP1A2, and had no effect on CYP2E1 in rat liver[47].

Table 2.1. CYP450 with the most known interaction potential.

CYP450 Isoform	Herbs (Induction)	Herbs (Inhibition)	Interactions Reported	References
CYP1A1	*Andrographis paniculata, Allium sativum, Salacia reticulata*	*Phyllanthus amarus, Curcuma longa, Berberis aristata, Carum carvi*	7	[41-45]
CYP3A4	*Glycyrrhizin glabra, Commiphora wightii*	*Myristica fragrans, Acorus calamus, Curcuma longa, Aesculus, indica, Centella asiatica, Andrographis paniculata*	7	[44, 46-52]
CYP1A2	*Allium sativum, Andrographis paniculata, Aesculus indica*	*Salacia reticulata, Berberis aristata, Curcuma longa*	6	[41, 45, 47, 53-55]
CYP2C9	-	*Myristica fragrans, Aesculus indica, Curcuma longa, Centella asiatica, Andrographis paniculata,*	5	[47, 48, 53, 56]

Most of the existing literature is exclusively based on reports on the synergistic actions of herbs and natural products with drugs and no reports on antagonism could be found. The reason might be poor reporting, as a patient may straight forwardly misconstrue an antagonism between both the chemical entities as to be an unproductive treatment. In many reported interactions, individual CYP enzyme contribution has not been described due to lack of suitable approaches for the identification for the antagonistic effect.

Table 2.2 summarizes some of the commonly used herbs and their natural products as perpetrators of CYP- and P-gp-mediated pharmacokinetic drug interactions. Evidences from *in vitro* and *in vivo* studies have indicated that herbs and their natural products can interact as inhibitors and/or inducer. For instance the bioavailability and half-life of propranolol were significantly enhanced by 2- and 3-folds, respectively, when used with *Allium sativum* [57]. *Allium sativum* is also reported to synergize the activity of glibenclamide and metformin [58, 59]. Similar interactions have been reported for *Andrographis paniculata*, wherein CYP2C9-mediated hydroxylation of tolbutamide was inhibited either by herbal extract or isolated constituent (andrographolide isolated from *Andrographis paniculata*) [60]. Activities of other DMEs have also been affected during concomitant use of various natural products. Ethanol extract of *Azadirachta indica* has been reported to increase the activity of aniline hydroxylase and inhibit the activity of NADPH cytochrome P450 reductase in RLM (rat liver microsomes) [61].

Table 2.2. Synergistic herb-drug interactions mediated through CYP enzymes.

Name of herb (Extract or natural products)	Interacting drug(s)	References
Allium sativum (Diallyl sulfide)	Ciprofloxacin, Amoxicillin, Ampicillin, Ritonavir, Warfarin, Glibenclamide, Saquinavir, Metformin, Propranolol	[57- 59]
Andrographis paniculata (Ethanolic extract)	Tolbutamide, Naproxen	[60]
Azadirachta indica (Seed oil extract)	Glibenclamide, Glipizide	[61]
Berberis aristata (Berberine)	Thiopental sodium Metformin, Tolbutamide	[62]
Cinnamomum verum (Bark extract)	Acarbose	[63]
Curcuma longa (Alcoholic rhizome extract)	Tolbutamide	[64]
Momordica charantia (Alcoholic fruit extract)	Rosiglitazone, Metformin, Chlorpropamide, Glibenclamide,	[65]

Table 2.2. Synergistic herb-drug interactions mediated through CYP enzymes…..*Continued*

Name of herb (Extract or natural products)	Interacting drug(s)	References
Morus alba (Leaf extract)	Metformin, Pioglitazone	[66]
Swertia chirata (Ethanolic extract)	Tolbutamide	[67]
Trigonella foenum - gracecum (Fenugreek seed, aqueous seed extract)	Phenytoin, Sulphonylureas	[68]
Acorus calamus (Hydroalcoholic extract)	Chloramphenicol, Cefuroxime, Tetracycline	[69]
Alpina galanga (Galangin, kaempferride and kaempferide-3-o-β-D-glucoside)	Amoxicillin	[70]
Anethum sowa (Carvone)	Gentamicin	[71]
Carum carvi (Butanolic seed extract)	Rifampicin	[72]
Cuminum cyminum (Aqueous seed extract)	Glyburide, Rifampicin	[73]
Glycyrrhiza glabra (Root extract, Glycyrrhizin)	Diphenhydramine, Nitrofurantoin	[74]
Plectranthus amboincus (Leaf oil extract)	Amikacin, Kanamycin, Gentamicin	[75]
Terminalia bellirica (Aqueous fruit extract)	Chloramphenicol, Ciprofloxacin, Tetracycline,	[76]
Wrightia antidysentrica (Ethanolic bark extract)	Novobiocin	[77]
Acorus calamus (Hydroalcoholic extract)	Sodium Valproate, Carbazepine	[78]
Centella asiatica (Ethanol and dichloromethane Extracts, asiaticoside and madecassoside)	Diazepam, Phenytoin, Gabapentin, Valproate	[79]
Enicostema axillare (Swertiamarin)	Phenytoin	[80]
Nardostachys jatamansi (Methanolic extract)	Phenytoin	[81]

While selected fatal case reports related to herbal medicines-modern drug interactions have been reported, the clinical pharmacokinetic studies on herbal medicines-modern drug

interactions are still inadequate, despite numerous opportunities for co-administration of herbs with modern allopathic drugs. In the ongoing discussion on the clinical relevance of potential herb-drug interactions, a recent report [82] of a fatality caused by mitragynine-quetiapine(QTE) interaction reminded researchers of deciphering this problem thoroughly. Overall, amid many case reports on this complex topic, clinical observations seem to be underreported and under-estimated. The reasons for the same could be as mentioned below:

- Most patients (~70%) do not inform their use of herbal medicines to their allopathic physicians
- Herbs have been used from ancient times, and laborious preclinical and clinical evaluations are not mandatory by regulatory authorities
- Most clinical trials of herbal formulations have incomplete value because of small sample size, poor design, and above all, the use of poorly defined formulations of undefined composition because of the absence of good quality controls.
- There is no complete investigation system for monitoring the adverse events of herbal formulations and herb-drug interactions in many countries.
- An herbal formulation usually contains many bioactive components, each of which may contribute to its pharmacological effects (pharmacodynamic and/or pharmacokinetic) and also drug interactions, leading to complications in predicting the mechanisms behind the observed herb-drug interactions.

However, understanding the prediction of pharmacokinetic drug interactions with natural products may help to avoid toxic or fatal herb-drug interactions in the clinic, if suitable experiments studies are designed and carried out using *in silico*, *in vitro* and *in vivo* natural product-drug interaction studies.

2.2. Anti-obesity Natural Products used for the Study

2.2.1. Hydroxycitric acid (HCA)

Garcinia cambogia, also known as Malabar tamarind is now commonly used as a component of weight-loss dietary supplements [83]. The pharmacodynamic effects of *G. cambogia* are closely related to its natural products constituents. The fruits of *G. cambogia* comprise organic acids, such as HCA (60%), xanthones (rheediaxanthone A, garbogiol), benzophenones (isogarcinol, garcinol) and amino acids such as γ-aminobutyric acid, glutamine and glycine [84]. Marketed supplements of *G. cambogia* extract frequently comprise 50-60% of HCA [85-87], which are mostly used for weight loss because of its hypolipidemic, anti-obesity and appetite-suppressant activities [88]. Indeed, plentiful clinical trials have reported effective

inhibitory effects of HCA on lipogenesis, reduction in serum triglyceride levels and on the adenosine triphosphate (ATP) citrate lyase, an important enzyme in the biosynthesis of fatty acids [89]. Additionally, boosted gluconeogenesis and glycogenesis have also been accredited to HCA in rats and mice [90]. Besides, in some non-clinical studies in rodents, *G. cambogia* fruit extracts have also been linked with appetite suppression activity, due to the increase in brain serotonin levels, inhibition of the enteral absorption of glucose and reduction in plasma insulin levels [91-92]. Table 2.3 shows a summary of various clinical studies conducted to understand the efficacy and safety of *G. cambogia* extract and HCA [85].

Table 2.3. Summary of clinical studies conducted on *G. cambogia* extract and HCA [85].

Study Design /Population /Duration	Treatment/Frequency of Dosing	Results
Parallel, randomized, double-blind, placebo-controlled /Over-weight and obese /6 weeks	*G cambogia* extract (750 mg), calcium (750 mg), guggulsterone extract (750 mg)/Daily	No significant differences between the groups
Parallel, randomized, double-blind, placebo-controlled /Over-weight and obese /8 weeks	*G cambogia* extract (1500 mg)+ chromium picolinate (300 μg)/Daily	No significant differences between the groups
Parallel, randomized, double-blind, placebo-controlled /Obese /8 weeks	*G cambogia* extract (1500 mg)/Daily	Higher significant weight loss in the *G cambogia* group *Vs.* placebo ($p < 0.05$)
Cross-over, randomized, double-blind, placebo-controlled /Normal weight to moderately obese /3 periods of 2 weeks	HCA (alone, 500 mg) or with 300 medium-chain Triglycerides/Daily	No significant differences between groups
Parallel, randomized, double-blind, placebo-controlled /Over-weight /12 weeks	*G cambogia* extract (3000 mg)/Daily	No significant differences between groups
Parallel, randomized, double-blind, placebo-controlled /Obese /12 weeks	*G cambogia* extract (300 mg)+ *Phaseolus vulgaris* (1200 mg)+ inulin (1200 mg)/Daily	Higher significant weight loss in the *G cambogia* group *Vs.* placebo
Parallel, randomized, double-blind, placebo-controlled /Overweight /12 weeks	*G cambogia* extract (2400 mg)/Daily	Higher significant weight loss in the *G cambogia* group *Vs.* placebo ($p < 0.05$)

Table 2.3. Summary of clinical studies conducted on *G. cambogia* extract and HCA [85] …. *Continued*

Study Design /Population /Duration	Treatment/Frequency of Dosing	Results
Parallel, randomized, single-blind, placebo-controlled/ Overweight and obese/8 weeks	Hydroxycitric acid (1000 mg)/Daily	No significant effects on weight between groups.
Parallel, randomized, double-blind, placebo-controlled/ Obese /8 weeks	*G cambogia* extract (396 mg)/Daily	Higher significant weight loss in the *G cambogia* group *Vs*. placebo ($p < 0.001$)
Cross-over randomized, single-blind, placebo-controlled/ Overweight and obese /2 periods of 2 weeks	Juice of tomato enriched with Hydroxycitric acid (900 mg) /Daily	No significant differences between groups
Parallel, randomized, double-blind, placebo-controlled/ Overweight and obese /12 weeks	*G cambogia* extract plus extracts of kidney bean pods, and chromium yeast /Daily	No significant differences between groups
Parallel, randomized, double-blind, placebo-controlled/ Overweight and obese /8 weeks	Hydroxycitric acid (2800 mg)alone or with niacin-bound chromium (4 mg) and *Gymnema silvestra* extract (400 mg)/Daily	Higher significant weight reduction in the *G cambogia* group alone or combined ($p < 0.001$)
Parallel, randomized, double-blind study/ Obese /12 weeks	*G cambogia* extract (2400 mg) plus *Amorphophallus konjac* extract (1500 mg)/Daily	No significant differences between groups

2.2.2. Quercetin (QCN)

Quercetin (3,3,4,5,7-pentahydroxyflavone, QCN) is ubiquitously present in many anti-obesity dietary supplements, and commonly used food and beverages like onion, black tea, red wine and various fruit juices [93]. QCN is reported to stimulate adenosine monophosphate-activated protein kinase (AMPK), and hence exerts anti-obesity activity. It inhibits CYP3A4 activity *in vitro* with an IC_{50} of 38 μM [94,95] and also inhibits P-gp to demonstrate increased bioavailability of P-gp and/or CYP3A4 substrates [96]. Data have shown that QCN interacts directly with transporter proteins to prevent drug efflux mediated by either BCRP or MDR1 or MRP1 (Table 2.4).

Table 2.4. Modulation of transporters by QCN [96].

Model systems (Cell lines/Animal Species)	**Victim Drug/Transporter Involved**
HCT-15 colon cells	Adriamycin/P-gp
MCF-7 ADR-resistant cells	Rhodamine 123/P-gp
KB-C2 cells	Daunomycin/P-gp
MCF-7/ADR cells	Daunomycin, Rodamine123/P-gp
KB-V1 cells	Vinblastine, Paclitaxel/P-gp
MCF-7 cells, MDA-MB 231 cells	Topotecan/P-gp
HeLa cells	Cisplatin/P-gp
HK-2 cells, Caco-2/VCR cells	Rhodamine 123/P-gp
Caco-2 cells	Cimetidine/P-gp
Glutathione S-transferase P1-1 (GSTP1-1) transfected	2,4-dinitrophenyl-S-glutathione (DNP-SG) /MRP1
Caco-2 cells	N-acetyl 5-aminosalicylic acid (5-AcASA) /MRP
Female SD rats	Tamoxifen/P-gp
Rats	Etoposide/P-gp
Rabbits	Diltiazem/P-gp
Male SD rats	Paclitaxel/P-gp
Male Yorkshire pigs	Digoxin/P-gp
Healthy volunteers	Fexofenadine/P-gp

It has been reported that QCN alters the activity of various phase I and phase II DMEs, including but not limited to CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C9, CYP2D6, CYP3A4, UDP glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) [97-103]. For example, QCN has been demonstrated to increase the bioavailability of several drugs, including ranolazine, paclitaxel, tamoxifen, valsartan and doxorubicin in rats; fexofenadine, cyclosporin A and rosiglitazone in humans; and digoxin in pigs [104-108].

2.2.3. Glycyrrhizin (GLZ)

Licorice combination of roots of *Glycyrrhiza uralensis, G. glabra* and *G. inflata*, is a very vital part of herbal medicine that has been extensively used due to its edible taste and therapeutic potential [109-111]. Glycyrrhizin (GLZ) is the key active component in licorice and it is reported that its content is between 2-15% (*w/w*) depending on the species, geographic and climatic conditions [112]. GLZ exerts numerous biochemical and pharmacological activities, including anti-obesity, anti-allergic, anti-inflammatory anti-viral and immuno-regulatory activities [113-115]. Besides, GLZ is thought to provide the characteristic sweetness and taste typically associated with licorice products [116], and therefore has been used extensively as a sweetening and flavoring agent in food and tobacco products [117].Glycyrrhetinic acid (GA), the aglycone of GLZ is also one of the active components of licorice. It occurs as two isomers: 18α-form and 18β-form [118,119]. The quantity of 18β-GA in licorice is found to be within 0.1-1.6% (*w/w*), whereas the quantity of 18α-GA is typically < 0.7% [118,119]. After oral intake, GLZ may be enzymatically hydrolysed to GA by colonic microflora [120-122] and might be responsible for the pharmacological properties of GLZ, including anti-viral and anti-inflammatory effects. GLZ and GA have been used for a diverse therapeutic purpose. Diseases like chronic hepatitis, are treated with chronic courses that may last for months and involve high doses of 200-300 mg/day of GLZ [123-124]. This is one important reason why GLZ and GA may end up being taken simultaneously with other allopathic drugs, and, therefore, probable interactions should be noted. CYPs are major categories of enzymes responsible for detoxification [125-128] and the effect of drugs on induction or inhibition of CYP450s is hence considered as one of the utmost criteria for potential interactions between drugs [129-134]. There have been reports on CYP induction by GLZ and are compiled in Table 2.5.

It has been recognized that PXR (pregnane X receptor) is a crucial regulator of CYP3A gene expression [135,136]. GLZ has shown to activate the DNA-binding capacity of PXR for the CYP3A4 element in response to xenobiotic signals. Both levels of mRNA and protein of CYP3A4 were amplified after the treatment of HepG2 cells with GLZ [135,136]. Therefore, it is most likely that the induction of liver CYP3A4 by GLZ is mediated through PXR activation.

Table 2.5. Induction of CYP3A by GLZ during *in vivo* studies [130-136].

Experimental model	Dose of Perpetrator	Effect on Victim Drug
Human	150 mg Monopotassium Glycyrrhizinate twice daily for 14 days	Midazolam: C_{max} ↓12%; AUC↓ 20%
Wistar rats	100 mg/kg/day GA Ammonium salt for 7 days	Male: ratio of AUC (Hydroxy-Midazolam to Midazolam)↑(0.87%)/(4.81%); Female: ratio of AUC (Hydroxy-Midazolam to Midazolam)↑(3.03%)/(7.61%)
Wistar rats	100 mg/kg/day GA Ammonium salt for 7 days	Triptolide: MRT↓ 35%; AUC↓72%; $t_{1/2}$↓29%
Male SD rats	150 mg/kg GLZ, 7 doses	Cyclosporine: C_{max} ↓ 49.0%; AUC_{0-t}↓45.6%
Human	150 mg GLZ salt tablet twice daily for 14 days	Omeprazole: Ratio of AUC ↓40%

Glucuronidation, an important conjugation reaction, plays a vital role in the removal of many endogenous components and clinical drugs and/or their metabolites. This reaction is mainly catalyzed by various UGT isoforms and, therefore, changes in the activity of UGT isoforms may impact the elimination of various xenobiotics and endogenous components [137,138]. The effects of GLZ and GA on the activity of UGTs are summarized in Table 2.6.

Table 2.6. Modulation of UGTs by GLZ and GA [139-140].

Experimental model/Compound	Concentration	Substrate	Effect
HLM/GA	20–80 mM	β -estradiol and SN-38	↑β-estradiol 3-glucuronidation; ↑SN-38 glucuronidation (catalyzed by UGT1A1)
HLM/GA	81 mg/mL	Morphine	↑Morphine 3-glucuronidation (catalyzed by UGT2B7)

There is a growing indication that some drug interactions may occur through the changes in the activity and/or expression of drug transporters. P-gp is an efflux transporter encoded by the MDR-1 gene in humans and is found in many organs [141]. Due to its (P-gp) broad substrate specificity, many studies have reported the effects of GLZ and GA on the activity of P-gp and are summarized in Table 2.7.

Table 2.7. Modulation of P-gp by GLZ and GA [142-144].

Experimental model/ Concentration or dose	Substrate	Effect
Human/ Monoammonium glycyrrhizinate (75 mg three times daily for 6 days)	Talinolol	No impact
Caco-2 cell/50 mM GA	[^{3}H] Digoxin	↑ apical-to-basolateral (A-B) transport of [^{3}H] digoxin; ↓basolateral-to-apical (B-A) transport of [^{3}H] digoxin
LLCGA5-COL150 cells/80.8 mM GA	[^{3}H] Digoxin	↓ [^{3}H] digoxin uptake
Male SD rats/18β-GA (16 mg/kg)	Digoxin	AUC of digoxin ↑ 51%; C_{max} ↑58%
KB-C2 cells/50 mM GA	Daunorubicin	↑ accumulation of daunorubicin

Considering the increasing use of HCA, QCN and GLZ as weight-loss natural products worldwide, it is essential to understand interactions between these natural products and other commonly used drugs to avoid probable harmful effects in terms of efficacy and safety.

2.3. Obesity and Psychosis

It is well known that obesity induces anxiety and depression and can quickly deteriorate the mental health condition of a person leading to severe psychosis. The predominance of obesity in schizophrenia has been reported as 1.5 to 4 times higher than the general population [145]. Schizophrenia-related weight gain and obesity might be either induced by medication or due to an intensified imbalance between energy intake and energy expenditure [146]. Similarly, bipolar disorder individuals are more frequently overweight (BMI 25.0 to 29.9), obese (BMI ≥ 30), or have a higher prevalence of central obesity, or both, compared with the general population [147]. Also, a study dealing with the meta-analysis confirmed a mutual relation between depression and obesity. Obesity has been found to increase the risk of depression, and depression was found to be predictive of developing obesity [148]. Quetiapine (QTE) is a frequently prescribed anti-psychotic drug with unique pharmacokinetic and pharmacodynamic properties, which makes it a treatment choice for several types of psychosis and bipolar disorder [149-152].

2.3.1. Quetiapine (QTE)

QTE, a dibenzothiazepine derivative is commonly prescribed to the patients with the exhibition of psychotic disorders. In human pharmacokinetic studies, QTE has been reported to be rapidly absorbed within 1 to 2 h after oral administration. It is ~ 83% bound to serum proteins and is eliminated primarily through hepatic metabolism with a mean terminal half-life of ~ 7 h [153]. *In vitro* studies show that QTE is mainly metabolised by CYP3A4. Other CYP isoforms perhaps do not contribute considerably to QTE metabolism. After administration of radioactive [^{14}C] QTE, ~ 73% of the radioactivity was recovered in the urine and 21% was recovered in faeces. Concurrent *in vivo* administration of QTE with the classical CYP3A4 inhibitor, ketoconazole, was found to increase systemic concentrations of QTE, with an increase in the mean C_{max} (2-folds) and AUC (5-folds) [154,155]. The significantly increased AUC and C_{max} was consistent with substantial first-pass metabolism and hepatic clearance of QTE through CYP3A4. Co-administration of QTE with carbamazepine, a known CYP3A4 inducer, led to a substantial reduction in the steady-state plasma concentrations of QTE. These results elucidate that concomitant administration of QTE with a strong CYP3A4 inducer could result in a significant increase in QTE metabolism and, hence possibly loss of clinical efficacy. Drug interactions that would markedly modify the plasma concentration of QTE are anticipated with strong CYP3A4 inducers or inhibitors [156]. Indeed, despite its broad spectrum of efficacy, QTE has some pharmacological disadvantages such as a narrow therapeutic range. This raises concern that further support the need to investigate the potential for pharmacokinetic-based interactions between commonly used anti-obesity natural products and QTE in *in-vivo* conditions.

2.4. Gaps in Existing Research

The majority of the herb-drug interaction studies are reported using herb extracts, while there are few studies on active natural products. An extract may have multiple constituents with variable compositions, so it is difficult to pin-point as to which natural products is responsible for the drug interaction. The existing research has not focused on detailed studies on active natural products such as HCA, QCN and GLZ dealing with understanding whether these compounds are substrates for any drug-metabolizing enzymes (DMEs) or transporters. Both P-gp and CYP3A4 are expressed on the villus tip of enterocytes, which is the primary site of absorption for drugs administered through the *p.o.* route. The modulation of both P-gp and CYP3A4 by natural products could significantly impact the bioavailability of co-administered drugs. To date, there is no study to understand this mechanism for the natural products selected for this research work. Also, HCA, 18β-GA (hydrolytic product of GLZ by intestinal bacteria) and QCN have the potential to undergo glucuronidation in the hepatocytes through UGTs. This

may further lead to drug interactions. Moreover, as bile acids are substrates of OATP1B1 and are glucuronidated in the hepatocytes too, one can expect competition for UGTs, which may also lead to increase levels of bile acids in plasma or hepatocytes. No studies have been reported to understand this phenomenon as well.

The current research may help to understand the impact of HCA, QCN and GLZ on the pharmacokinetics of QTE. It may also help us to understand the impact on bio-molecule disposition like bile acids, which may lead to hepatotoxicity. Overall, the metabolite identification and reaction phenotyping of the three active natural constituents has not been reported. Also, no inhibition studies of various drug-metabolizing enzymes for these constituents are known. Based on these experiments, one should be able to clearly understand whether HCA, QCN and GLZ have the tendency to act as a victim or perpetrator. Also, no *in vivo* studies are reported for the above selected natural products to understand their pharmacokinetic interaction potential with QTE.

2.5. Aim

Considering the potential gaps in the existing research, the present thesis aims at 'Assessment of Anti-Obesity Natural Products for Pharmacokinetic Based Interaction with Quetiapine: *In-silico, in-vitro and in-vivo* studies' and involve an array of studies that include information on HCA, QCN and GLZ and their interaction potential with QTE, a commonly used anti-psychotic modern drug.

2.6. Objectives

- To perform *in silico* metabolite profiling of HCA, QCN, GLZ and GA using Gastroplus® software
- To develop and validate UPLC-MS/MS based bio-analytical methods for HCA, QCN, GLZ, GA and QTE
- To perform *in vitro* metabolite profiling and metabolic stability studies of HCA, QCN, GLZ and GA using UPLC-HRMS
- To evaluate the *in vitro* inhibition/induction of hepatic enzymes by HCA, QCN, GLZ and their impact on QTE clearance in rat liver S9 using UPLC-MS/MS.
- To conduct *in vivo* drug interaction pharmacokinetic studies of HCA, QCN and GLZ with QTE

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