CONCLUSION, FUTURE SCOPE AND LIMITATIONS

CHAPTER 7

7. Conclusion, Future Scope and Limitations

7.1. General conclusion

Obesity (body mass index, BMI $\geq$ 30 kg/m^2) is the single largest risk factor for non-communicable diseases like cardio-vascular complications, cancer, diabetes mellitus, etc and these diseases account for more than 2/3rd of early deaths worldwide. Generally, the drugs approved for obesity are intended to be used for patients with a BMI of $\geq$ 30 (obese) or $\geq$ 27 (overweight) with an associated risk factor, e.g., diabetes and/or hypertension.

Natural products based dietary supplements for weight loss include a wide variety of products and are available in different dosage forms including but not limited to capsules, liquids, tablets, bars and powders. These natural products can play an effective role in treating obesity specially those containing polyphenols, flavonoids, sterols, alkaloids etc. Generally, these natural products are believed to be 'safe' and deprived of side effects as they are mostly of plant origin and are often constituents of food or dietary supplements. However, the combined use of herbs/natural products with allopathic drugs may decrease, increase or mimic the effects of either constituent, which may result in interactions with these drugs. Examples include renowned clinical case is St. John's Wort that has led to a decrease in the area under the curve (AUC) of a variety of clinical drugs, including the ones with low therapeutic range, *e.g.,* cyclosporine and warfarin.

It is well known that obesity induces anxiety and depression and can quickly deteriorate the mental health of a person leading to severe psychosis and bipolar disorders. Obesity has been found to increase the risk of depression, and depression is predictive of developing obesity. In the ongoing discussion on the clinical relevance of potential herb/natural productd-drug interactions, a recent report of a fatality caused by Mitragynine-Quetiapine (QTE) interaction has again reminded researchers of interpreting this problem thoroughly.

Accordingly, commonly used anti-obesity natural products that are used as/in popular dietary supplements, i.e., hydroxycitric acid (HCA), glycyrrhizin (GLZ) and quercetin (QCN) were studied for the pharmacokinetic interaction potential with quetiapine (QTE), an allopathic drug usually prescribed for the treatment of psychosis, depression and anxiety. Various i*n silico*, *in vitro* and *in vivo* studies were designed to decipher the mechanism of interactions.

Our *in silico* studies using ADMET® predictor (Simulations Plus, USA) and *in vitro* profiling studies using freshly isolated rat liver S9 (RLS9) fraction indicated that QCN has a high propensity to metabolism and it metabolises primarily through conjugation reactions. HCA

underwent a minimal metabolism and GLZ completely converted to glycyrrhizetinic acid (GA) through hydrolysis, which further experienced conjugation metabolism.

UPLC-MS/MS (ultra-performance liquid chromatography-tandem mass spectrometry) based bio-analytical methods were developed and validated as per US FDA guidelines. These methods were used for quantitative estimation of HCA, QCN, GLZ, GA and QTE from *in vitro* (rat liver S9 fractions) and *in vivo* (rat brain homogenate, rat plasma) samples. The developed methods were sensitive, selective, fast, accurate and precise. For the first time, an UPLC-MS/MS based bio-analytical method for pharmacokinetics and bioavailability of HCA acid from marketed *Garcinia* preparation in rats is being reported. Similarly, a simple method for simultaneous estimation of QCN, GLZ, GA and QTE through use of polarity switching in UPLC-MS/MS is reported for the first time.

Our *in vivo* studies in Wistar rats indicated that no significant changes were observed on the pharmacokinetics of QTE upon co-administration with HCA. However, QCN could significantly alter the pharmacokinetic profiles of QTE by changing the activity of CYP3A4 and P-gp. A significant increase (4-5 folds) in C_{max} and $AUC_{(0\text{-last})}$ of QTE upon QCN pre-treatment was observed. The brain to plasma ratio was increased by 2.5 folds in the animals exposed to pre-treatment with QCN as compared to the animals pre-treated with vehicle. On the other hand, GLZ significantly decreased the C_{max} and $AUC_{0\text{-last}}$ and increased the clearance of QTE. The brain to plasma ratio was 0.88 in rats exposed to pre-treatment with GLZ and 3.04 for the animals pre-treated with vehicle.

Significant increase in the levels of QTE in plasma and brain, when co-administered with QCN raises concern about herb-drug interactions potentially leading to toxicity. Hence, QTE doses may require special attention if used along with QCN containing herbs/dietary supplements to avoid the complications due to the increased bioavailability. This is even more concerning, as QTE has a very narrow therapeutic window and sub-therapeutic concentrations poses substantial risk to patients, as it might increase bipolar disorder and suicidal tendency. On the other hand, induction of drug metabolising enzymes and efflux transporter (CYP3A4/P-gp) by GLZ might increase the metabolism and thereby clearance of QTE from the body. Thus, patients receiving QTE should be alerted when dietary supplements containing GLZ are used for long-term. Further therapeutic drug monitoring may be required to institute guidelines for concomitant use of GLZ and QTE.

In conclusion, the studies presented in this thesis indicated that the natural products that are present in various anti-obesity dietary supplements interact with various drug metabolizing enzymes either as substrates, inhibitors and/or inducers. Also, it is evident that natural

products-drug interactions have important clinical and toxicological implications, and severe testing for possible drug interactions with widely used natural products is need of the hour.

7.2. Specific conclusions

7.2.1. Chapter - 1

- Obesity is the single largest risk factor for non-communicable diseases like cardio-vascular complications, cancer, diabetes mellitus, etc and these diseases account for more than 2/3rd of early deaths worldwide
- Between the years 1975 and 2014, the obesity (BMI ≥ 30 kg/m^2) prevalence has increased from 3.2% to 10.8% in adult men and from 6.4% to 14.9% in adult women
- The dietary supplements for weight loss include a wide variety of products and are available in many different dosage forms including but not limited to capsules, liquids, tablets, bars and powders
- Various botanicals, along with their active constituents and probable mechanism of action were discussed
- A brief introduction to pharmacokinetics has also been included in this chapter

7.2.2. Chapter – 2

- A comprehensive literature review (relevant to present study) was performed on the topic of herb-drug interactions including details on individual natural products
- The literature highlighted that there is a potential gaps in the existing research on drug interaction studies with anti-obesity natural products
- The aim and objectives were detailed considering the potential gaps in the research

7.2.3. Chapter - 3

- The materials including chemicals, animals, instruments, software and liquid chromatography columns employed in the study were detailed
- The methods of preparation of rat liver S9 fraction (RLS9) and subsequent incubation of hydroxycitric acid (HCA), glycyrrhizin (GLZ), and quercetin (QCN) in the RLS9 were discussed
- Details of method development using tandem mass spectrometry and high-resolution mass spectrometry along with the Pharmacokinetics studies for the selected natural products were covered

7.2.4. Chapter - 4

- *In silico* studies was performed using the ADMET Predictor®
- For *in vitro* studies, compounds were incubated in freshly isolated rat liver S9 fractions.

- QCN underwent through high metabolism and a total of 31 metabolites were observed when it was incubated in freshly isolated RLS9 fraction
- HCA exhibited minimal metabolism and only one direct glucuronidated metabolite was observed (This is the first report on the metabolism of HCA)
- GLZ was completely converted to GA, which further underwent phase II metabolic reactions and yielded glucuronidated, di-glucuronidated, acetylated and sulphated metabolites

7.2.5. Chapter - 5

- Part A of the chapter dealt with bioanalytical UPLC-MS/MS method development and validation for HCA and its application in a pharmacokinetic study were presented
- The method was fast (run time: 5 minutes), sensitive (LLOQ: 10.5 ng/mL), and highly selective
- Part B of the chapter dealt with simultaneous bioanalytical method development for QCN, GLZ, GA and QTE using UPLC-MS/MS in rat plasma.
- The developed method was sensitive (LLOQ for QCN, GA and GLZ: 1 ng/mL and QTE: 0.1 ng/mL), rapid (run time: 2.5 minutes), and efficient for the quantification of these compounds in rat plasma.
- The proposed methods were time and resource saving and were validated as per USFDA guidelines

7.2.6. Chapter - 6

- This chapter dealt with the impact of HCA, QCN and GLZ on the pharmacokinetics of QTE
- The results indicated no significant change in the pharmacokinetics of QTE in the animals pre-treated with HCA as compared to the vehicle group.
- Hence, no clinically relevant pharmacokinetic-based interactions can be expected for this combination
- QCN notably increased the systemic exposure of QTE and decreased the clearance in accordance with the *in vitro* results
- A significant increase in $AUC_{(0\text{-last})}$ (627.98 ± 80.33 (QCN treated) *Vs.* 120.64 ± 25.47 ng/mL*h (vehicle treated) of QTE upon QCN pre-treatment was observed
- GLZ notably decreased the systemic exposure of QTE ($AUC_{0\text{-last}}$ (72.25 ± 18.57 (GLZ treated) *Vs.* 120.64 ± 25.47 ng/mL*h (vehicle treated) and increased the clearance in accordance with the *in vitro* results
- Induction of CYP3A4/P-glycoprotein might accelerate the metabolism/clearance of QTE

from the body

- Patients receiving QTE should be alerted when herbal dietary supplements containing QCN and GLZ

7.3. Future Scope and Limitations

7.3.1. Future Scope

The present thesis has resulted in understanding of anti-obesity natural products and their pharmacokinetic interaction with Quetiapine. These results can stimulate further studies of other commonly used anti-obesity natural products with various classes of drugs consumed by obese population. These results can also be helpful in the future clinical investigations of cases arising due to such herb/natural product-drug interactions. Herb-drug interactions are difficult to characterize and resolve, because of the lack of comprehensive regulatory framework around safety, efficacy, and manufacturing standards for herbal/dietary products. Therefore, regulations on anti-obesity medicinal herbs/natural products/ dietary supplement and herb-drug interactions would be required. Appropriate regulations for adverse events monitoring and labelling requirements of natural products to alert consumers for possible interactions with other concomitantly used drugs would be desirable. This would enable accurate product labelling and provide a knowledge base on potential herb-drug interactions to medical professionals.

7.3.2. Limitations

The set of studies present in this thesis were designed and executed to understand metabolism based pharmacokinetic interactions of anti-obesity natural products with commonly used anti-psychotic drug, quetiapine. While, a significant body of knowledge has been generated through developing novel bio-analytical methods and understanding of the natural product-drug interactions through *in silico*, *in vitro* and *in vivo* pre-clinical studies, human studies were not part of this project. For any potential herb-drug/natural product-drug interactions, well-designed clinical studies with reasonable sample size are required to confirm the interaction, however, these studies are time-consuming and expensive.