ABSTRACT

Obesity is a chronic, relapsing progressive disease. Severe changes in the global food system combined with sedentary life style have resulted in a rapid upsurge in energy consumption, transforming obesity into pandemic dimensions over the past 50 years. Moreover, the comorbid factors associated with chronic obesity including but not limited to psychotic disorders, hypertension, diabetes, etc. have placed the global obese population under a greater risk of mortality. Herbal or natural products can play a safe and effective role in treating obesity specially those belonging to polyphenols, flavonoids, sterols, and alkaloids. Obesity and Psychotic disorder have been found to show a positive correlation especially with respect to the metabolic disturbances. Natural anti- obesity agents have been used by patients suffering from psychosis. However, use of these anti-obesity natural/herbal products along with commonly used anti-psychotic drugs could lead to potential life-threatening pharmacokinetic drug interactions mainly mediated through drug metabolizing enzymes or transporters. Recently, a report of fatality caused by natural product Mitragynine due to interaction with antipsychotic drug Quetiapine (QTE) prompted us to construe the drug interaction potential of widely used anti-obesity natural products with QTE.

The aim of the current research work was to study the pharmacokinetic interaction potential of anti-obesity natural products namely hydroxycitric acid (major active constituent of *Garcinia cambogia,* HCA), glycyrrhizin and glycyrrhizetinic acid (major active constituents of *Glycyrrhiza glabra,* GLZ and GA) and quercetin (common flavonoid present in many food items, such as, onion, grapes, berries and citrus fruits, QCN) with commonly used anti-psychotic agent QTE.

In silico studies using ADMET® predictor from GastroPlus® (Simulations Plus, USA) and *in vitro* metabolite profiling were performed on QCN, GLZ and GA. The results indicated that QCN had a high propensity to metabolism and suggest that QCN metabolises primarily through conjugation reactions instead of phase I reactions. These results correlated well with *in vitro* studies, wherein 31 metabolites were observed when QCN was incubated in freshly isolated rat liver S9 (RLS9) fraction. HCA underwent a minimal metabolism and only one direct glucuronidated metabolite was observed when incubated in RLS9. This is the first report on the metabolism of HCA. GLZ completely converts to GA through hydrolysis, which further experienced conjugation metabolism.

UPLC-MS/MS (ultra-performance liquid chromatography-tandem mass spectrometry) based bio-analytical methods were developed through selection of internal standards, stationary phase, mobile phase, organic modifier, detector and further 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 (lower limit of quantification of 10.5 ng/mL for HCA; 1 ng/mL for GLZ, GA and QCN; and 0.1 ng/mL for QTE), selective, fast (run-time of $\leq$ 5 minutes), accurate and precise. Moreover, a simple one-step, reproducible and cost-effective extraction procedure through protein precipitation (*via* use of 10% trichloro acetic acid in water for HCA and 100% acetonitrile for all the other analytes) was developed. For the first time, an UPLC-MS/MS based bio-analytical method for pharmacokinetics and bioavailability of HCA from marketed *Garcinia* preparation in rats is being reported. The method was successfully applied to determine *in vivo* pharmacokinetics of HCA, that suggested that HCA is rapidly absorbed with a moderate apparent volume of distribution (0.6 L/kg) and has a good bioavailability (~82%). Similar bioavailability of HCA was observed after the administration of marketed formulations of *G. cambogia* (~61%). The method reported herein can be used routinely in any bioanalytical laboratory for analysis of HCA and could be helpful for further scientific evaluation of *Garcinia* products. Also, for the first time a simple method for simultaneous estimation of QCN, GLZ, GA and QTE through use of polarity switching in UPLC-MS/MS is being reported. The simultaneous method was successfully applied to a pharmacokinetic study of QCN, GLZ, and GA following *p.o*. administration of QCN and GLZ to rats (Dose: 10 mg/kg) and for all the pharmacokinetic interaction studies.

Based on the *in vivo* studies in Wistar rats, 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 in C_{max} (92.07 ± 12.15 *Vs.* 20.48 ± 4.91 ng/mL) and $AUC_{(0\text{-}last)}$ (627.98 ± 80.33 *Vs.* 120.64 ± 25.47 ng/mL*h) of QTE upon QCN pre-treatment was observed. The brain to plasma ratio was 7.73 in the animals exposed to pre-treatment with QCN and 3.04 for the animals pre-treated with vehicle. The post hoc test further revealed that in QCN treated group, the levels of QTE metabolism were significantly reduced in the RLS9. 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. To date, this is the first report about the effects of QCN on the absorption and disposition of QTE.

In case of GLZ, the *in vivo* studies demonstrated that a 7-day pre-treatment with GLZ

remarkably decreased the C_{max} (10.05 ± 2.21 *Vs.* 20.48 ± 4.91 ng/mL) and $AUC_{0\text{-}last}$ (72.25 ± 18.57 *Vs.* 120.64 ± 25.47 ng/mL*h) and increased the clearance of QTE that correlated well with *in vitro* results. The brain to plasma ratio was 0.88 in rats exposed to pre-treatment with GLZ and 3.04 for rats pre-treated with vehicle. Induction of drug metabolising enzymes and efflux transporter (CYP3A4/P-gp) might increase the metabolism and thereby clearance of QTE from the body. Thus, patients receiving QTE should be alerted when herbs/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.

Evidence from our *in silico, in vitro* and *in vivo* studies has indicated that the natural products present in the herbs/dietary supplements interact with various drug metabolizing enzymes either as substrates, inhibitors and/or inducers, and it is apparent that the modulation of these enzymes by such natural products is complex and depends upon type of herb/natural product, their route of administration, dose, etc. These pharmacokinetic interactions are not confined to the liver, but may also occur in other tissues where the drug metabolizing enzymes and/or drug transporters are considerably expressed, in particular in the gastrointestinal tract, as medicinal herbs/natural products are most often given orally. Generally high concentrations (more than IC_{50}) of the administered herbs/natural products are expected to be present in the gastrointestinal tract, thereby impacting the absorption of co-administered drugs. Moreover, the multiple natural products in herbs may modify the intestinal pH and motility, and inhibit and/or induce intestinal drug transporters such as P-gp, and thus change the rate and extent of concomitant drug absorption.

To conclude 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. It is perhaps time to consider herbs and their natural products not as alternative medicine based on tradition and experience, but as phyto-therapy, and an integrated part of modern medical treatment. Safety (e.g. herb-drug interactions), quality and efficacy of herbal/dietary supplements should be proved, based on an objective and appropriate standard as for modern drug discovery.