(KRBC/PL) were independent of the initial rat blood concentrations of FMN. The bioavailability of unchanged/free FMN was found to be poor, i.e. approximately 3%. FMN was found to have a high clearance (5.13 L/h/kg) and a large apparent volume of distribution (14.16 L/kg). Circulating conjugates (glucuronides/sulfates) of FMN and daidzein (DZN) were quantified using enzymatic hydrolysis of plasma samples. The levels of isoflavone glucuronides/sulfates were found to be much greater than that of the corresponding aglycones.

> Intravenous Pharmacokinetics and Oral Bioavailability of Biochanin A in Rats

The oral bioavailability of BCA was found to be low (4.6%) and this may be due to extensive first-pass metabolism by phase I oxidative metabolism and phase II glucuronidation and/or sulfation in the intestine as well as in the liver. The BCA was extensively converted to its metabolites genistein (GEN), GEN and BCA conjugates (glucuronides and/or sulfates). BCA was found to have a high clearance (15.70 L/h/kg) and a large apparent volume of distribution (10.56 L/kg).

3. Interaction studies:

A significant number of people regularly consume dietary supplements, hoping to improve their health and prevent or fight certain diseases. However, many ingredients in the dietary supplements are not thoroughly studied and may cause severe interactions with certain drugs. In the present study we are reporting effect of co-administration of biochanin A (BCA), formononetin (FMN) and kaempferol (KMF) on pharmacokinetics of tamoxifen, raloxifen and centchroman requiring chronic administration.

> Interaction study of BCA with tamoxifen

BCA co-administration significantly (p< 0.05) decreased area under the plasma concentration-time curve from time zero to time infinity (AUC0-∞) and peak tamoxifen concentrations (Cmax). Consequently, the relative bioavailability (RB%) of tamoxifen coadministered with BCA was remarkably decreased compared to the control group. The AUC0-∞ and Cmax of 4-hydroxytamoxifen in BCA pretreated rats were also significantly (p<0.05) lower than those from control group. If the results of this study are further confirmed by clinical trials, tamoxifen dosages should be adjusted to avoid potential drug interaction.
Interaction study of BCA with raloxifen

Pretreatment with BCA at 100 mg/kg, significantly (p<0.05) increased the AUC₀⁻∞ (36.50 %) in comparison to control. Consequently, the absolute bioavailability (AB %) of raloxifen in the presence of BCA was remarkably increased (1.36 fold) compared to the control. The Cₘₐₓ of raloxifen was increased non significantly upon pretreatment with BCA (42.29 %, 51.26 % and 66.06 % for 20, 50 and 100 mg/kg, respectively). If the results of this study are further confirmed by clinical trials, raloxifen dosages should be adjusted to avoid potential drug interaction when raloxifen is used clinically in combination with BCA and BCA-containing dietary supplements.

Interaction study of BCA with centchroman

BCA co-administration has induced no significant changes in pharmacokinetic parameters of centchroman. Therefore, we can conclude that BCA and BCA containing dietary/herbal supplements can be administered safely with centchroman.

Interaction study of KMF with centchroman

The pretreatment of KMF significantly (P<0.05) increased the peak concentrations (Cₘₐₓ, 139.41 % and 241.36% increase for 20, and 50 mg/kg of KMF, respectively) and area under the plasma concentration-time curve (AUC₀⁻∞, 41.46% and 194.22% increase for 20, and 50 mg/kg of KMF, respectively) of centchroman. If the results obtained from the rats’ model is confirmed in the clinical trials, the centchroman dose should be adjusted for potential drug interactions when centchroman is used with BCA or the BCA-containing dietary supplements.

Interaction study of KMF with raloxifen

KMF co-administration has induced no significant changes in pharmacokinetic parameters of raloxifen. From the finding of present study we can conclude that KMF and KMF containing dietary/herbal supplements can be administered safely with raloxifen.
Interaction study of FMN with tamoxifen

FMN co-administration at 50 mg/kg significantly (p<0.05) increased the AUC_{0-\infty} (40.20 %) and C_{max} (68.96 %) of orally administered tamoxifen. Consequently, the relative bioavailability (RB%) of tamoxifen in the presence of FMN is remarkably increased (40.20 %) compared to the control. FMN at 50 mg/kg, significantly (p<0.05) increased the AUC_{0-\infty} (66.12 %) and C_{max} (65.86 %) of 4-hydroxytamoxifen in comparison to control group. If the results obtained from the rats' model is confirmed in the clinical trials, the tamoxifen dose should be adjusted for potential drug interactions when tamoxifen is used with FMN or the FMN-containing dietary supplements.

Interaction study of FMN with centchroman

FMN co-administration has induced no significant changes in pharmacokinetic parameters of centchroman.

Interaction study of FMN with raloxifen

FMN co-administration has induced no significant changes in pharmacokinetic parameters of raloxifen.

4. CYP Inhibition Study

The purpose of present study was to assess the CYP inhibition potential of flavanoids, kaemferol (KMF), biochanin A (BCA) and formononetin (FMN) which are principle components of many marketed herbal/dietary supplements.

FMN has not shown significant inhibitory effect on any of the CYP isoforms examined and appears to be relatively safe while co-administering with drugs.

BCA also has not shown significant inhibitory effect on most of the CYP isoforms except CYP3A with IC50 value of 66.82 μM and 52.18 μM for nifedipine and testosterone as probe substrate, respectively.

KMF appears to be moderate inhibitor for CYP2C9, CYP2C19, CYP2E1 and CYP3A4, and weak inhibitor of CYP1A2. However, it has not shown significant inhibitory effect on CYP2D6. Inhibitory effect of KMF on major CYPs isoforms arise major safety concerns regarding its co-administration with drugs metabolised by same CYP isoforms which may
result in potential drug-drug interaction and alteration of pharmacokinetics of many medications.

5. Standardization of Permeability (PAMPA and In-situ) Models:

➤ **Standardization of Parallel artificial membrane permeability assay (PAMPA)**

Parallel artificial membrane permeability assay (PAMPA) was introduced as a rapid and cost effective method to determine passive drug permeability across a phospholipid system. The PAMPA model was successfully standardized using known permeability markers (low and high permeability markers) and used successfully for permeability determination of test compounds.

➤ **Standardization of In-situ Permeability Model**

The rat in situ intestinal perfusion is a commonly used technique for the assessment of permeability of drugs and new chemical entities. The effective permeability coefficient values of marker compounds obtained in rats were compared with published data for human intestinal permeability ($P_{\text{eff (human)}}$) and human fraction absorbed ($\text{Fa (human)}$) to establish in-house model. Strong correlations were found between rat and human values for markers ($P_{\text{eff (human)}} = 1.039 P_{\text{eff (rat)}} - 0.1815; R^2 = 0.970$ and $\text{Fa (human)} = 0.1562\ln (P_{\text{eff (rat)}}) + 0.7232; R^2 = 0.927$). Considering the high correlation of rat $P_{\text{eff}}$ values with those of human reported values, we conclude that the developed in-house model is reliable and can be used preliminarily, to predict human permeability and fraction dose absorbed of any test compound.