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Effect of sex and food on the pharmacokinetics of different classes of BCS drugs in rats after cassette administration

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ABSTRACT

The cassette dosing technique is employed in the drug discovery stage of non-clinical studies to obtain pharmacokinetic data from multiple drug candidates in a single experiment. The objective of the current investigation was to evaluate the effect of sex and food on the selected pharmacokinetic parameters of four biopharmaceutical classification system (BCS) drugs (BCS-I: propranolol, BCS-II: diclofenac, BCS-III: atenolol, and BCS-IV: acetazolamide) utilizing cassette dosing in male and female rats under fed and fasting conditions. Different animal groups were dosed intravenous (i.v) and oral at 1 and 10 mg/kg, respectively, in the form of cassette at a dose of 5 mL/kg. Blood samples were analyzed by liquid chromatography-tandem mass spectrometry. Pharmacokinetics parameters were calculated using Phoenix software version 8.1. A significant increase ($p < 0.05$) of the area under the plasma concentration–time ($AUC_{0\text{-last}}$) was observed for diclofenac and acetazolamide in females over males after i.v dosing. Additionally, acetazolamide showed greater instantaneous concentration at the time of dosing, and clearance in females ($p < 0.05$) compared to males after i.v administration. After oral dosing, propranolol exhibited significant variations ($p < 0.05$) in the maximum drug concentration (C_{max}), $AUC_{0\text{-last}}$, the volume of distribution (V_d), and bioavailability in females as compared to males under fed state. Diclofenac showed significant changes ($p < 0.05$) in $AUC_{0\text{-last}}$, and clearance (Cl) in females as compared to males under fasting and fed state. However, acetazolamide exhibited a significant enhancement ($p < 0.05$) in $AUC_{0\text{-last}}$, V_d, and Cl in fasting females than the males. The data here illustrates that there is an appreciable difference in AUC and C_{max} values exist in male and female rats under fed and fasting conditions administered with the cassette dosing of tested BCS class drugs.

1. Introduction

Various in-vitro assays are conducted to estimate the absorption, distribution, metabolism, and excretion (ADME) of new chemical entities (NCEs) before animal testing (Jacob and Nair, 2018). Despite the availability of many in-vitro methods, animal experiments continue to remain as a gold standard to assess the preclinical pharmacokinetic parameters. Cassette pharmacokinetics is a proven non-clinical method for the selection of druggable compounds, amongst multiple novel chemical entities in a single experiment, although different approaches are available for conducting drug disposition (Manitpisitkul and White, 2004). Cassette dosing technique was introduced in 1990 by Berman et al with low expense and lesser number of animals (Nagilla et al., 2011; Watanabe et al., 2006). Cassette dosing is one of the best methods used for the rapid assessment of comparative pharmacokinetics of multiple candidates during the drug discovery process (White and Manitpisitkul, 2001). Typically, this study involves simultaneous dosing of 5–10 compounds instead of individuals in a group of animals. Thus, this technique achieves all required pharmacokinetics information of different drugs with fewer animals in a single study (Nagilla et al., 2011).

It is generally believed that there are potential drug-drug interactions during cassette study, which could alter the pharmacokinetics of NCEs (Smith et al., 2007). The possible drug-drug interactions and

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thereby alteration in the pharmacokinetics reported were mainly noticed in compounds tested with similar structure and follows identical metabolic pathways. Additionally, in the oral route, there could be an interaction associated with absorption and first-pass metabolism (Alqahtani et al., 2018). However, according to our knowledge, no studies have been carried out so far to delineate the occurrence of drug-drug interactions using compounds from all four classes of the biopharmaceutics classification system (BCS). In the meantime, the BCS classification has a significant role in clinical drug development (Cook et al., 2008). Solubility, intestinal permeability, and dissolution rate are the major factors affecting bioavailability according to the BCS classification (Chaudhary et al., 2021; Shekhawat PB, Pokharkar VB, 2017; Tambosi et al., 2018).

Propranolol, diclofenac, atenolol, and acetazolamide belong to four different BCS classes. Indeed, these four drugs have different characteristics, attributes and are regularly used in clinical practices (Benet et al., 2011). Propranolol belongs to BCS class I with high solubility, high permeability (Lindenberg et al., 2004). It is a non-selective β-blocker, which is capable of blocking the action of epinephrine on both β_1 and β_2 adrenergic receptors (Asdaq and Inamdar, 2011). It is metabolized by cytochrome P450 CYP1A2 and inhibits CYP2D6 (Kastelova and Yanev, 2002). Diclofenac is a cyclooxygenase inhibitor, belongs to BCS class II (Van Den Abeele et al., 2016), and is used to treat inflammation and pain as a result of inflammation (Chohan et al., 2019). This drug is primarily metabolized by CYP2B6, involving CYP2C9, and has slight mechanism-based inhibition of CYP3A4 (Tang, 2003). With low permeability and high solubility, atenolol belongs to BCS class III and generally shows high variation in the rate and extent of drug absorption (Anroop et al., 2009; Nair et al., 2009). This selective β_1 blocker does not have CYP-based metabolism and is excreted unchanged through the kidney (Yang et al., 2007). Acetazolamide is classified as BCS class IV due to its low solubility and low permeability and is mostly excreted through the renal route (Ghadi and Dand, 2017). A review of the literature indicated that so far CYP related data are unavailable for acetazolamide (Hampson et al., 2016).

It is a well-known fact that food can affect the pharmacokinetics of drugs (Briguglio et al., 2018). On the other hand, various natural differences exist between males and females of all species, and these changes in the body are more prominent after the development of reproductive organs. Usually, these changes lead to variation in anatomy, physiology, biochemistry, and pathophysiology of species (Franconi and Campesi, 2014; Nicolas et al., 2009; Soldin et al., 2011). It is well-known that concomitant use of multiple drugs potentially increases the drug-drug interactions in patients (Carpenter et al., 2019). Thus, both food and sex may also influence the pharmacokinetics of different drugs during cassette dosing. A review of literature also signifies that there is no detailed study carried out in terms of the effect of sex and food on the pharmacokinetic parameters of various BCS classes of drugs by cassette dosing. Taking these points into consideration, the objective of this study was to assess the effect of sex and food on the selected pharmacokinetic parameters of different BCS class drugs obtained by cassette dosing in rats. The 3Rs (replacement, reduction, and refinement) technique is an animal welfare policy widely practiced in research to treat laboratory animals. This 3Rs practice was employed to assess the pharmacokinetics of propranolol, diclofenac, atenolol, and acetazolamide dosed intravenously and orally using cassette dosing in male and female *Sprague Dawley* rats in fed and fasting states. To follow the 3R practice, we replaced (1R) the individual dosing method with cassette dosing technique, which considerably reduced (2R) the number of animals required, and used the venous cannulation technique to minimize the pain, stress, and sufferings (refinement-3R) to animals and thus improves their overall well-being.

2. Materials and methods

2.1. Materials

Propranolol, diclofenac, atenolol, acetazolamide, methylcellulose, solutol HS15, dipotassium ethylenediaminetetraacetic acid (K_2EDTA), and telmisartan were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Pre-coated K_2EDTA microtubes were purchased from PreQ (Mumbai, India). HPLC grade acetonitrile, methanol, water, and formic acid were purchased from Merck specialties Pvt Ltd (Mumbai, India). Silicon tubing for cannulation was procured from Bio-Sci Instruments (Mumbai, India).

2.2. Animals

The study protocol (B-011) was approved by the Institutional Animal Ethics Committee (IAEC) and Committee for Control and Supervision of Experiments on Animals (CPCSEA-1125/PO/Rc/S/07/CPCSEA). *Sprague Dawley* rats (age: 8–9 weeks) (18 numbers, 8 males and 8 females) with an average weight of 280 g for males and 250 g for females were procured from Hylasco Bio-Technology Pvt Ltd. (Hyderabad, India) and were subjected to three days' quarantine period in American Association for Accreditation of Laboratory Animal Care and Office of Laboratory Animal Welfare accredited vivarium. Animals were maintained and monitored for good health following test facility standard operating procedures and at the discretion of the laboratory animal veterinarian. Normal rodent pellet feeds (Safe Diet, Samitek Instruments, New Delhi, India) and water were given ad libitum. Environmental controls for the animal room were set to maintain a temperature of 22 ± 2 °C, relative humidity of 40–70%, and a 12-h light/12-h dark cycle. Normal healthy animals certified by the laboratory animal veterinarian were selected and acclimatized for a minimum of three days before initiation of the study. Animals were randomized into 6 groups, 3 groups each for female and male, while an individual group comprised of 3 animals. For oral administration, animals were fasted overnight for a duration of 12 h before dosing with ad libitum water.

2.3. Rat jugular vein cannulation

Rats were anesthetized using isoflurane at 5% in the chamber during cannulation, and anesthesia was maintained with 2% isoflurane using isoflurane dispensing assembly (EZ Anaesthesia Machine, Palmer, PA, USA). A cannula of silicone tubing with an internal diameter of 0.75 mm and an outer diameter of 1.25 mm was used to cannulate the animals. The right jugular vein was exposed, a loose ligature was placed caudally, and the cranial end of the vein was ligated. A small incision was made between the ligatures into which the catheter was inserted. The catheter was secured in place by fastening the loose ligature around the catheterized vessel. A small incision was made in the scapular region to serve as the exit site of the catheter. The catheter was subcutaneously tunneled and exteriorized through a scapular incision. A stay suture was tied in the scapular area. The incision was sutured with sterile suturing material. The surgical procedure was performed on a temperature-controlled heating surgical pad. An anti-septic solution was applied to the sutured site of the rat and scapular region. Patency was tested, and the catheter was filled with a lock solution (heparinized saline) and sealed with a stainless-steel plug. Animals were closely observed throughout the recovery period (Feng et al., 2015).

2.4. Pharmacokinetics

The details of the study plan are summarized in Table 1. Dosing and bleeding of animals were performed according to standard protocols (Nair et al., 2018). Animals were dosed at 1 mg/kg with a dose volume of 5 mL/kg intravenously and 10 mg/kg with a dose volume of 5 mL/kg orally via intra-gastric gavage. Feed was provided 4 h post-dosing for

Table 1
Details of the pharmacokinetics of the study plan.

Study variables	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Animal species/ strain/ (body weight)	Rat/ Sprague Dawley (200–300 g)					
Test item	Propranolol, diclofenac, atenolol and acetazolamide as cassette					
Sex	Male	Female	Male	Male	Female	Female
Number of animals	3	3	3	3	3	3
Route of administration	Intravenous		Peroral			
Feeding condition	Fed	Fed	Fasting	Fed	Fasting	Fed
Dose (mg/kg)	1	1	10	10	10	10
Dose volume (mL/kg)	5	5	5	5	5	5
Concentration (mg/mL)	0.2	0.2	2	2	2	2
Formulation vehicle	DMSO (10%) + Solutol (10%) in WFI (90%)		Tween 80 (1%) + methylcellulose (99 %)			
Anti-coagulant	K_2EDTA and heparin pre- coated tubes	Lithium-heparin pre-coated tubes				
Timepoints	Intravenous: 5, 15, 30 min, 1hr, 2hr, 4hr, 8hr, and 24hrOral: 15, 30 min, 1hr, 2hr, 4hr, 8hr and 24hr					

DMSO; dimethyl sulfoxide, K_2EDTA; dipotassium ethylenediaminetetraacetic acid, WFI; water for injection.

fasted groups. Animals were not fasted for intravenous formulation, prepared in dimethyl sulfoxide (10% v/v) + solutol (10% v/v) in water for injection (90% v/v). Oral formulation was prepared using Tween 80 (1% v/v) + methylcellulose (99% v/v). Animals were randomly separated into 6 groups and individual groups consist of three animals (n = 3). Equal numbers (n = 9) of male and female rats constituted the total test animals in the current investigation. Rats of Group 1 (male, fed) were dosed intravenously with cassette solution of propranolol, diclofenac, atenolol, and acetazolamide. In intravenous administration absorption process is absent and therefore including both fasting and fed conditions will have little impact on serum drug concentration–time profile. Similarly, in Group 2 (female, fed) animals were dosed intravenously. Group 3 (male, fasting), Group 4 (male, fed), Group 5 (female, fasting), and Group 6 (female, fed) were dosed orally with the cassette suspension of drugs. The first two drops of blood were discarded from the cannula to ensure the removal of any excess heparinized saline before the collection of blood. The external catheter was connected to a syringe, in which 0.20–0.25 mL of blood was collected and transferred to a pre-labeled tube. The cannula was filled with heparinized saline and sealed. Blood samples collected were stored on ice, before centrifugation. Centrifugation was conducted at 4000 rpm for 10 min at 4 °C. The plasma samples were separated and transferred to pre-labeled microcentrifuge tubes and promptly frozen at –80 ± 10 °C until bioanalysis.

2.5. Bioanalytical method

2.5.1. Preparation of calibration and quality control samples

Stock solutions of propranolol, diclofenac, atenolol, acetazolamide and telmisartan (internal standard) were prepared in dimethyl sulfoxide (DMSO) at 1 mg/mL concentration. Working standard solutions were prepared by serial dilution from master stock in DMSO: methanol: water (20:40:40, v/v) proportions, respectively. Working standard solutions were prepared at 25-fold higher concentrations to achieve the final extracted blood calibration and quality control samples concentrations. A total of nine calibration standards and three quality control samples of all the above standards were prepared individually. Calibration standards for propranolol, diclofenac, atenolol, and acetazolamide were with the lower limit of quantification level (LLOQ) of 1.18, 1.12, 1.02 and 1.03 ng/mL, and upper limit of quantification values of 1174.34, 1117.2, 1019.2, and 1029.6 ng/mL, respectively. The quality control samples (laboratory quality control; 4.55, 4.33, 3.95 and 3.99 ng/mL, mid-quality control; 569.11, 541.41, 493.92 and 498.96 ng/mL, high-quality control; 948.51, 902.35, 823.2 and 831.6 ng/mL) of all the standards were prepared in blood by spiking individually 1 µL of the working standard solution into 24 µL of blank plasma. The working solution of internal standard (200 ng/mL) was prepared by dilution of an aliquot of master stock solution with acetonitrile. All the standards and telmisartan solutions were stored at 4 °C in polypropylene tubes.

2.5.2. Sample preparation

A 25 µL aliquot of plasma (samples from the pharmacokinetic study) were transferred into a 96 well polypropylene plate and samples were precipitated with 200 µL acetonitrile containing internal standard at 200 ng/mL Samples were vortexed for 5 min at 1000 rpm and centrifuged at 4000 rpm for 5 min at 4 °C. The supernatant (110 µL) was separated and transferred into a fresh plate for analysis and diluted with 110 µL of methanol: water (1:1, v/v), from this 5 µL was injected for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

2.5.3. LC-MS/MS analysis

All mass spectrometric detections were performed on the QTRAP 5500 instrument (SCIEX) with a Turbo V^{TM} ionization source interface. Data acquisition and processing for quantification were performed using Analyst software version 1.6.3 (SCIEX). The mass spectrometric conditions were optimized for the compounds by infusing a 500 ng/mL solution in methanol: water (1:1, v/v) at 10 µL/ min flow rate using an infusion pump (Harvard Apparatus, Holliston, USA) connected directly to the mass spectrometry. Flow-dependent source parameters were optimized by flow injection analysis with 1 mL/min, a flow rate of mobile phase without column. The Turbo V source with electrospray ionization probe was operated with optimized settings as follows: polarity: positive, curtain gas: 40 psi, nebulizer gas: 50 psi, heater gas: 55 psi, ion spray voltage: 5500 V, source temperature: 550 °C. The mass spectrometry was operated in MRM mode in which both parent ion and fragment ion are fixed. The *m/z* value of propranolol, diclofenac, atenolol, acetazolamide parent, and fragment ions used was 260–157, 296.1–215, 267.2–208, and 223.1–181 with optimum declustering potential 110, 70, 100, 70 V and collision energy of 25, 25, 24, 19 V, respectively. For telmisartan 515.4 and 276.120 were the *m/z* values used for parent and fragment ions with declustering potential and collision energy of 130, 45 V, respectively.

The LC system used was SciexExion consisting of two isocratic pumps, a vacuum degasser, and a temperature-controlled AD Multi-Plate Autosampler set at 4 °C and a thermostatic column oven set at 40 °C. The stationary phase used for the chromatography was Kinetex EVO, C18 with 5 µm particle size and dimensions of 50 × 4.6 mm (Phenomenex, Torrance, CA, USA). The mobile phase consisting of 10 mM ammonium acetate with 0.1% formic acid in water (aqueous reservoir) and 100% acetonitrile (organic modifier) was used at a flow rate of 1.0 mL/min. A common reverse-phase gradient programme [time (min)/ % B = 0.01/2, 0.5/50, 1.00/90, 2.60/90, 2.70/2, 3.7/2] was used with a short run time of 3.7 min. Representative LC-MS/MS chromatograms of extracted blank, standard and internal standard are presented in Fig. S1.

2.5.4. Selectivity and specificity

The selectivity and specificity of the method were evaluated by analyzing the chromatograms of blank rat plasma collected without

administration of the drug. Further, the plasma samples were spiked with propranolol, diclofenac, atenolol, acetazolamide, and internal standards were analyzed for any interference of co-eluting peaks in the chromatogram of plasma. The purity of all four drugs peaks in plasma was also verified. No interfering peaks were detected at the analyte retention time.

2.5.5. Linearity

The linearity of propranolol, diclofenac, atenolol, acetazolamide over nine points in the working standards was prepared by spiking 50 μL of drug-free rat plasma with sufficient amount of propranolol, diclofenac, atenolol, acetazolamide, and internal standard. These samples were subjected to the above-mentioned sample preparation to get concentration profiles of all four drugs in plasma in the range of 1 to 1000 ng/mL. The concentration of internal standard was maintained at 200 ng/mL The samples were analyzed in triplicate, and a calibration curve was constructed by plotting concentration against peak area ratio of analyte to the internal standard. Good linearity (R2 greater than 0.99) was observed for all the drugs in the range (1 to 1000 ng/mL).

2.5.6. Accuracy and precision

The method was evaluated for accuracy and precision by analysis of quality control samples at four different concentration levels within the calibration range (1 ng/mL, 4 ng/mL, 500 ng/mL, and 840 ng/mL) in plasma. The freshly prepared samples were analyzed in five replicates on the same day for inter-day, and the same samples were analyzed on three consecutive days, for intraday. Briefly, a specific quantity of the drug was added to the known concentration of plasma samples. Then the drug was extracted using the above-mentioned procedure and the samples were analyzed by optimized LC-MS/MS procedure. The quantity recovered from plasma was estimated using respective regression equations. The accuracy was calculated as percent recovery and precision as percent relative standard deviation, and was found to be excellent and was in the range.

2.5.7. Recovery study and matrix effect

For studying the effectiveness of extraction of propranolol, diclofenac, atenolol, acetazolamide in plasma, its recovery study was performed. All four drugs were spiked in blank plasma having standard concentration at low, middle, and upper levels and samples were determined by the LC-MS/MS bioanalytical method developed. Then, other solutions were prepared without biomatrix and spiked with the same standard concentration. The area ratio of propranolol, diclofenac, atenolol, acetazolamide peak to internal standard peak in samples with and without biomatrix was obtained. The recovery was determined using the following formula:

$$\%\ \text{Recovery} = \frac{[\text{Peak area ratio of spikedanalytein plasma sample}] \times 100}{[\text{Peak area ratio ofanalytewithout plasma}]}$$

The effect of the matrix in the quantification of all four drugs at the LLOQ level was compared with matrix and without matrix response. The data signifies a negligible matrix effect on the recovery.

2.5.8. Stability of propranolol, diclofenac, atenolol, acetazolamide in plasma

The propranolol, diclofenac, atenolol, acetazolamide stability was assessed in rat plasma using different storage and handling conditions of samples. The stability of all four drugs in plasma samples was kept for 6 h at room temperature (25 °C) and then analyzed to determine the effect for the short-term excursion. In another stability study, the analyzed sample was kept in the auto-sampler for 24 h at 5 °C and again analyzed the processed samples, for determination of the auto-sampler stability. Finally, a freeze–thaw study was conducted, a plasma sample containing all four drugs was stored for 4 h at −20 °C, after a specified time interval, the sample withdrew from the freezer and thawed at room temperature, and kept for 1 h outside. Then, the samples were again kept in the freezer and repeated this freeze–thaw cycle three times and samples were analyzed. The stability of all four drugs in plasma samples was determined with obtained results and compared with the standard concentrations of propranolol, diclofenac, atenolol, acetazolamide. The data indicates the good stability of drugs in both temperatures.

2.6. Pharmacokinetic parameter analysis

Pharmacokinetic parameters were calculated for the individual rat plasma concentration by using a non-compartmental model, linear log trapezoidal (200–202), with Phoenix software version 8.1. Parameters calculated in intravenous were as follows; C_o (ng/mL) (predicted concentration at the time of dose), $T_{1/2}$ (h) (terminal half-life), V_d (L/kg) (volume of distribution), Cl (mL/min/kg) (total body clearance), $AUC_{0\text{-}last}$ (ng.h/mL) (area under the plasma concentration–time curve zero to last time point), $AUC_{0\text{-}inf}$ (ng.h/mL) (area under the plasma concentration–time curve zero to extrapolated infinite time point), $MRT_{0\text{-}last}$ (h) (mean residence time) (Nair et al., 2012). Whereas for oral (maximum concentration achieved throughout the duration) C_{max} (ng/mL), (time to reach maximum drug concentration) T_{max} (h), and other main parameters like (volume of distribution based on the terminal phase) $V_{z\_}$ obs (L/kg), (clearance based on the c_{last}) Cl_obs (mL/min/kg), $AUC_{0\text{-}last}$ (ng·h/mL), $AUC_{0\text{-}inf}$ (ng·h/mL), $MRT_{0\text{-}last}$ (h) and %F (bioavailability) were calculated [$\%F = ((AUC_{PO}/AUC_{IV})*(DOSE_{IV}/DOSE_{PO}))*100$] (Jhaveri et al., 2020). The plasma concentration in certain cases was measured only up to 4 or 8 h, as no drug level was detected or were below the limits of quantification thereafter. Hence, the AUC in these cases was calculated according to the last time point mentioned.

2.7. Statistical analysis

The data are expressed as median (range) unless otherwise stated. The statistical comparison of experimental data between the groups was performed using the Mann-Whitney *U* test (GraphPad Prism software version 5, San Diego, CA, USA). The difference in values at $p < 0.05$ is considered statistically significant.

3. Results

3.1. Intravenous

Concentration-time profiles of propranolol, diclofenac, atenolol, and acetazolamide after intravenous cassette administration to male and female *Sprague Dawley* rats in fasted and fed conditions at 1 mg/kg was depicted in Fig. 1. The observed pharmacokinetic parameters are summarized in Table 2. It is evident from Fig. 1 and Table 2 that there was no difference in pharmacokinetic parameters between males and females for propranolol and atenolol after intravenous administration. Nevertheless, a significant increase (161%; $p < 0.05$) in $AUC_{0\text{-}last}$ was noticed in females related to males for diclofenac (Table 2). Similarly, a significant increase (47%; $p < 0.05$) in $AUC_{0\text{-}last}$ was observed in females as compared to males for acetazolamide (Table 2).

3.2. Oral

The concentration–time profiles of propranolol, diclofenac, atenolol, and acetazolamide after oral cassette administration to male and female rats in fasting and fed condition at 10 mg/kg was depicted in Figs. 2-5, respectively. The observed pharmacokinetic parameters in different sexes and food are summarized in Tables 3 and 4, S1 and S2. Comparison of AUC and C_{max} are displayed in Figs. 6 and 7, respectively. In general, the data signifies that the female rats (fasting or fed) showed higher bioavailability than the male counterparts (Fig. 6). Comparatively, a similar observation was also noticed with the C_{max} values in all the four BCS class drugs studied (Fig. 7). However, no significant differences in the bioavailability were noticed in male rats (fasting vs fed) for all four

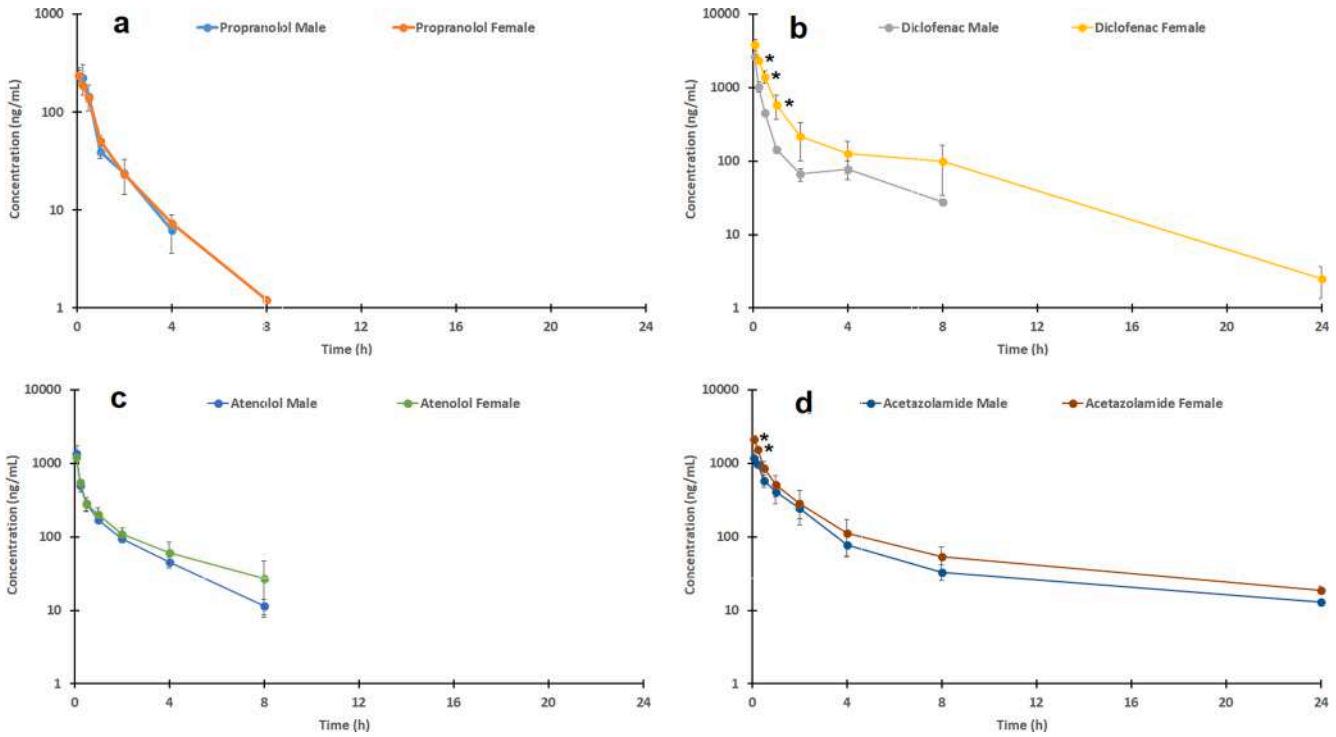

Fig. 1. Concentration-time profiles of (a) propranolol, (b) diclofenac, (c) atenolol, and (d) acetazolamide after intravenous cassette administration to male and female *Sprague Dawley* rats in fed condition at 1 mg/kg. Drug level in the plasma was below detectable at 24 h for propranolol male, diclofenac male, atenolol male and female. The data presented are mean with SD error bars (n = 3) and pharmacokinetic parameters analyzed by the Mann-Whitney *U* test. *Statistically significant ($p < 0.05$) from male group.

category drugs (Table S1). In addition, when the results were analyzed to assess the relationship between the effects of food and physico-chemical properties, it was found that when the log P value of the drug decreases, the absorption also decreased in the fed state.

3.2.1. Propranolol (BCS Class-I)

A significant increase in $AUC_{0\text{-last}}$ (197%; n = 3; $p < 0.05$) was noticed in fasting females as compared to fasting males (Table 3 and S3; Fig. 6) and a similar observation was noticed in the case of fed condition wherein, the fed female $AUC_{0\text{-last}}$ was about 83% more (statistically insignificant) than that of fed male (Table 4). Significantly higher ($p < 0.05$) percentage bioavailability (%F) was recorded in females (122%) as compared to males (43%) in fasting condition (Table 3). Similarly, %F was found to be more in females (67%) as compared to males (38%) in the fed condition, although statistically insignificant (Table 4). There is no or very little bioavailability difference in fasting v/s fed by class 1 drug for males (Table S1) whereas for females 83% decrease was noticed in fed over the fasting condition (Table S2; Fig. 6). The V_d and Cl were found significantly high ($p < 0.05$) in fasting males as compared to the fasting females (Table 3).

3.2.2. Diclofenac (BCS Class-II)

A significant increase in $AUC_{0\text{-last}}$ (100%; n = 3; $p < 0.05$) was noticed in fasting females as compared to fasting males (Table 3; Fig. 6). However, no change in bioavailability was estimated in fasting females (%F; 112.14%) v/s fasting males (%F; 137.35%) (Table 3). In the case of the fed condition, 53% improvement ($p < 0.05$) in $AUC_{0\text{-last}}$ was revealed, while the bioavailability was also changed (141.24% fed, male and 88.59% fed, female) (Table 4). There was a non-significant effect between fasting v/s fed on class II drug for $AUC_{0\text{-last}}$ in males (Table S1) but in females, there was a 27% decrease ($p < 0.05$) in $AUC_{0\text{-inf}}$ in fed condition (Table S2). The V_d was found comparable in both fasting and fed conditions in males and females (Tables S1 and S2). However, the Cl was significantly higher ($p < 0.05$) in both fasting and fed conditions in males as compared to their female counterparts (Tables S1 and S2).

3.2.3. Atenolol (BCS Class-III)

A statistically insignificant increase of $AUC_{0\text{-last}}$ (47%; n = 3) and bioavailability was noticed in fasting females (%F; 123%) as compared to fasting males (%F; 94%) (Table 3; Fig. 6). No change in $AUC_{0\text{-last}}$ among males and females was noted in the fed condition (Table 4). However, the observed $AUC_{0\text{-last}}$ values in the fed condition were lesser than fasting in both males (102%) and females (145%; $p < 0.05$) (Tables S1 and S2). No significant variation in the V_d or Cl values was noticed for males and females in both fasting and fed conditions (Tables 3 and 4).

3.2.4. Acetazolamide (BCS Class-IV)

A significant increase of $AUC_{0\text{-last}}$ (108%; n = 3; $p < 0.05$) and bioavailability was noticed in fasting females (%F; 43%) as compared to fasting males (Table 3; Fig. 6). No significant change in $AUC_{0\text{-last}}$ was noticed for males in fasting and fed conditions (Table S1). However, a significant decrease ($p < 0.05$) in $AUC_{0\text{-last}}$ in fed condition for females (122%; $p < 0.05$) was observed when compared with fasting females (Table S2). The V_d and Cl were also found significantly high ($p < 0.05$) in fasting males as compared to the fasting females (Table 3).

4. Discussion

Cassette dosing is an improvised technique for the fast selection of NCEs, based on pharmacokinetic studies. This technique can drastically reduce the expenses and time for the selection of novel candidates (Nagilla et al., 2011; White and Manitpisitkul, 2001). However, many investigations indicate that there is a chance of drug-drug interaction during clinical cassette dosing (Yoshikado et al., 2017). On the other hand, both BCS and biopharmaceutics drug disposition classification systems (BDDCS) are considered complementary systems intended for the rapid development of drug molecules (Cook et al., 2008). In general,

Table 2
Pharmacokinetic parameters of propranolol, diclofenac, atenolol, and acetazolamide after cassette intravenous administration in fed *Sprague Dawley* rats (n = 3) at 1 mg/kg.

Drug	Propranolol- BCS (Class-I)		Diclofenac- BCS (Class-II)		Atenolol- BCS (Class-III)		Acetazolamide- BCS (Class-IV)	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
C_0 (ng/mL)	265.57 (193.37–273.71)	263.74 (221.05–310.50)	4347.08 (3593.13–5158.50)	5301.25 (3472.12–5765.08)	2269.08 (1594.09–2913.46)	1792.26 (1723.39–1878.00)	1437.91 (881.36–1698.98)	2371.60 (2245.55–2977.22)*
$T_{1/2}$ (h)	1.184 (0.90–1.26)	1.29 (1.08–1.48)	3.18 (2.95–3.64)	3.27 (2.21–3.63)	1.87 (1.85–2.02)	2.12 (1.82–7.28)	9.29 (7.27–10.06)	7.65 (7.56–13.44)
K_{el} (h^{-1})	0.59 (0.55–0.77)	0.54 (0.47–0.64)	0.22 (0.19–0.24)	0.21 (0.19–0.31)	0.37 (0.31–0.37)	0.33 (0.095–0.38)	0.075 (0.069–0.095)	0.091 (0.052–0.092)
V_d (L/kg)	7.90 (6.55–8.87)	8.43 (7.07–10.11)	3.39 (2.76–3.62)	1.30 (0.87–1.97)	3.25 (3.00–3.40)	3.00 (2.76–6.95)	7.07 (4.32–8.22)	3.72 (3.15–7.77)
Cl (mL/min/kg)	86.56 (60.22–101.44)	75.83 (75.57–78.78)	11.48 (10.83–12.31)	4.61 (4.53–6.29)	20.06 (15.71–21.22)	15.06 (11.01–19.10)	8.11 (6.87–10.21)	5.62 (4.81–6.67)*
$AUC_{0\text{-last}}$ (ng. h/mL)	182.86 (159.20–260.21)	208.80 (207.63–218.42)	1312.97 (1228.51–1411.44)	3434.66 (2642.82–3598.71)*	803.99 (758.93–1013.91)	995.74 (835.00–1051.57)	1855.02 (1474.88–2290.96)	2730.26 (2175.54–3259.55)
$AUC_{0\text{-inf}}$ (ng.h/mL)	192.54 (164.30–276.77)	219.79 (211.56–220.56)	1452.17 (1353.52–1539.28)	3614.56 (2651.67–3678.16)*	830.79 (785.28–1060.57)	1106.35 (872.47–1512.94)	2054.62 (1631.57–2427.54)	2963.66 (2496.99–3464.75)*
AUC_{Extra} (%)	5.03 (3.10–5.99)	1.30 (0.97–5.53)	9.24 (8.30–9.59)	0.44 (0.33–6.62)*	3.35 (3.23–4.40)	4.95 (4.29–34.19)	9.60 (5.63–9.71)	7.88 (5.92–12.87)
$MRT_{0\text{-last}}$ (h)	0.87 (0.76–0.88)	1.10 (0.92–1.23)	1.36 (1.36–1.57)	2.05 (1.51–3.97)	1.43 (1.42–1.56)	1.68 (1.53–2.33)	4.06 (4.04–4.43)	3.86 (3.86–5.08)

All the values are mentioned as Median (Range) and analyzed by the Mann-Whitney *U* test. Cassette solution was dosed intravenously at a dose of 1 mg/kg each compound. C_0: Predictive concentration at the time of dosing, $T_{1/2}$: half-life, V_d: Volume if distribution, Cl: clearance, $AUC_{0\text{-last}}$: area under the curve for all blood time points. $AUC_{0\text{-inf}}$: area under the curve from time zero extrapolated to infinite time, $MRT_{0\text{-last}}$: mean residence time. *Statistically significant ($p < 0.05$) from the male group.

the main goal of BCS is to identify the drug molecules for biowaiver, while BDDCS predicts both drug disposition as well as drug-drug interactions (Benet, 2013). Thus, the selection of compounds based on these classes may be advantageous to avoid drug-drug interactions. Moreover, in-vitro data can be a good tool where the physicochemical properties (solubility and permeability) can be considered for the selection of compounds for cassette dosing. Drug-drug interaction mainly occurs in three ways; a) by making complex with each other, b) by occupying the absorption site of each other and c) by inhibiting and inducing the CYP enzyme of each other (Lynch and Price, 2007; Palleria et al., 2013).

If the physiochemical properties (e.g. solubility, permeability) of all compounds selected in the cassette are different, then there will be fewer chances of interaction by complexation. Based on the solubility differences, various compounds were selected belonging to individual BCS classes. However, interaction is possible if the absorption site is the same for more than one compound. Although this will occur in the case of very high doses and preclinical pharmacokinetics, however, in humans a comparatively very low dose is used. In addition, the permeability of all the four compounds studied is different. It is also reminded that interaction could be by CYP inhibition and induction (Gupta et al., 2013). None of the four compounds studied have overlapping inhibition and induction of the CYP.

The bioavailability of a drug is influenced by various factors including low absorption, high metabolism, and clearance from the body (Satyavert et al., 2021). Comparison of various pharmacokinetic parameters (V_d, Cl, AUC, and MRT) of all four drugs tested after oral and intravenous administration in both male and female fed rats signifies higher AUC in oral (Table 2 and Table 4), due to the higher oral dose (10 mg/kg) compared to intravenous (1 mg/kg). The propranolol, diclofenac, atenolol, and acetazolamide could be classified as moderate to the high apparent volume of distribution compounds by considering normal value as 0.7 L/kg and the observed data (7.90 and 8.43 L/kg, 3.39 and 1.30 L/kg, 3.25 and 3.00 L/kg, 7.07 and 3.72 L/kg for male and females, respectively). MRT was found higher in the case of acetazolamide and moderate for the remaining three compounds for intravenous administration (Table 2). The Cl was higher for propranolol whereas the other three compounds could be classified as low clearance compounds after intravenous administration, and it was comparable in the case of diclofenac and acetazolamide (low solubility drugs). In the case of oral administration, the MRT was higher in atenolol and moderate for the remaining three drugs in both fast and fed conditions (Tables 3 and 4). The Cl was found higher in propranolol and atenolol, while it was comparable for diclofenac and acetazolamide.

Physiological differences such as body weight, mass index, water content, surface area, etc. result in pharmacological and pharmacokinetic differences between males and females (Gandhi et al., 2004; Soldin and Mattison, 2009). Pharmacological variations are also important factors to be considered. For instance; V_d is smaller in females as compared to males, however, the free fraction is more in females when compared with males while the clearance from the body is slower (Whitley and Lindsey, 2009). Drug–receptor interactions may be modified by changes in receptor sensitivity, this being influenced by complex regulatory and homeostatic factors. Physiologic differences among men and women influence drug response, besides pharmacokinetics and pharmacodynamics. Pharmacodynamic differences in women demonstrate greater sensitivity to drugs and enhanced efficacy of beta-blockers, opioids, selective serotonin reuptake inhibitors, and typical antipsychotics (Whitley and Lindsey, 2009). Additionally, a high percentage of women (50–75%) are prone to experience an adverse drug reaction compared to men.

Excipients have also been shown to exhibit sex-specific differences in humans and animals, boosting drug bioavailability in men but not women through the action of an excipient. A human study with excipient PEG 400 at pharmaceutically relevant concentrations significantly increased cimetidine bioavailability in men and not women ($p < 0.05$)

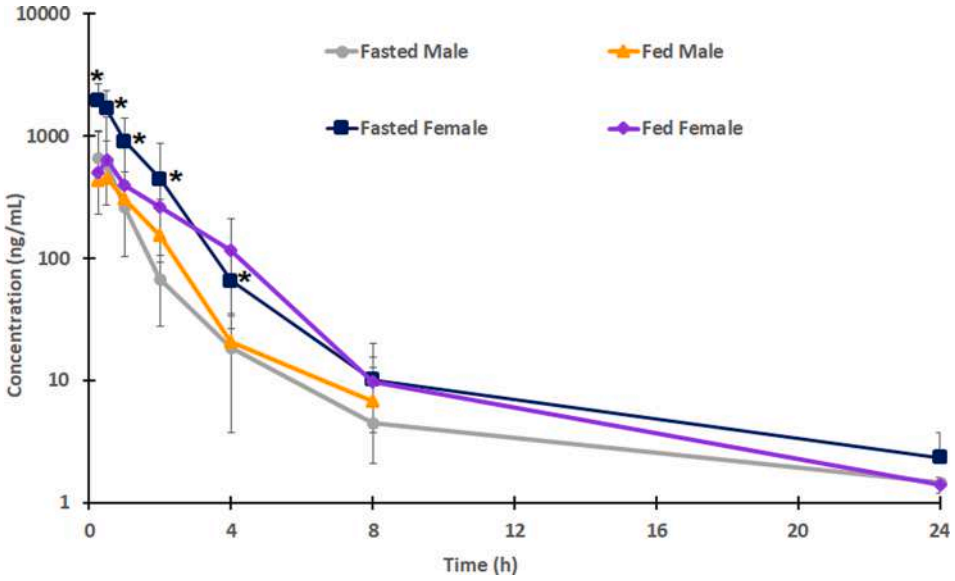

Fig. 2. Concentration-time profiles of propranolol after peroral cassette administration to male and female *Sprague Dawley* rats in fasting and fed condition at 10 mg/kg. The data presented are mean with SD error bars (n = 3) and pharmacokinetic parameters analyzed by the Mann-Whitney *U* test. *Statistically significant ($p < 0.05$) from male group.

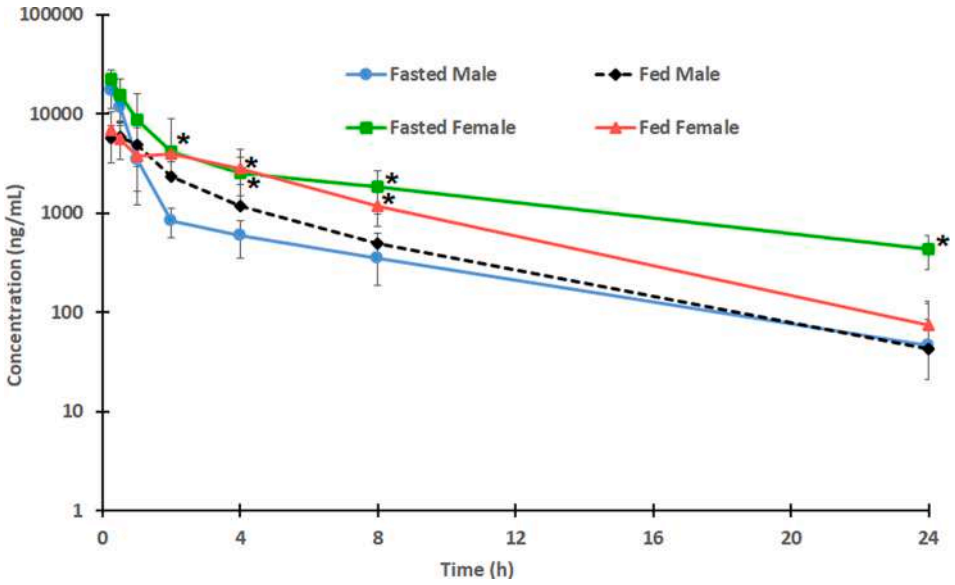

Fig. 3. Concentration-time profiles of diclofenac after peroral cassette administration to male and female *Sprague Dawley* rats in fasting and fed condition at 10 mg/kg. The data presented are mean with SD error bars (n = 3) and pharmacokinetic parameters analyzed by the Mann-Whitney *U* test. *Statistically significant ($p < 0.05$) from male group.

(Mai et al., 2020). Such sex- and the dose-dependent pharmacological effect was found to be due to its modulatory effect on intestinal P-gp. It was reported that polyoxyethylated excipients such as PEG 2000, Cremophor RH 40, Poloxamer 188, and Tween 80 were capable of reducing the activity and expression of intestinal P-gp (Mai et al., 2019), resulting in the enhancement in ranitidine bioavailability in male rats, but not in females. It was demonstrated that non-polyoxyethylated excipient, Span 20 enhanced bioavailability in both male and female rats in a non-sex-dependent manner. This study signifies the requirements for the screening of inactive pharmaceutical ingredients in terms of their selection and use for oral drug product development. An observable difference between the fasted and fed states in both sexes of Wistar rats along the stomach and intestinal segments of the gastrointestinal tract have been reported (Dou et al., 2020). In addition, the efflux membrane transporter P-gp displayed a significant sex difference ($p < 0.05$) in the proximal small intestine, the main sites of drug absorption among male and female rats in both the fasted and fed state. Sex differences in therapeutic outcome and toxicity are usually linked to physiological differences, which influence oral bioavailability, Cl, and V_d. The interplay between metabolizing enzymes and efflux has positioned drug transporters such as Pgp as an important mediator of sex variability at the molecular level (Bebawy and Chetty, 2009). Mechanistically, P-gp controls serum drug concentration due to its drug efflux capacity, therefore serves as a significant contributor to both the oral and biliary clearance of many of its drug substrates.

In addition, the gastric pH among males (1.92) and females (2.59) are also different resulting in varying unionized fractions of weak acids and weak bases (Soldin and Mattison, 2009). In the current study, the

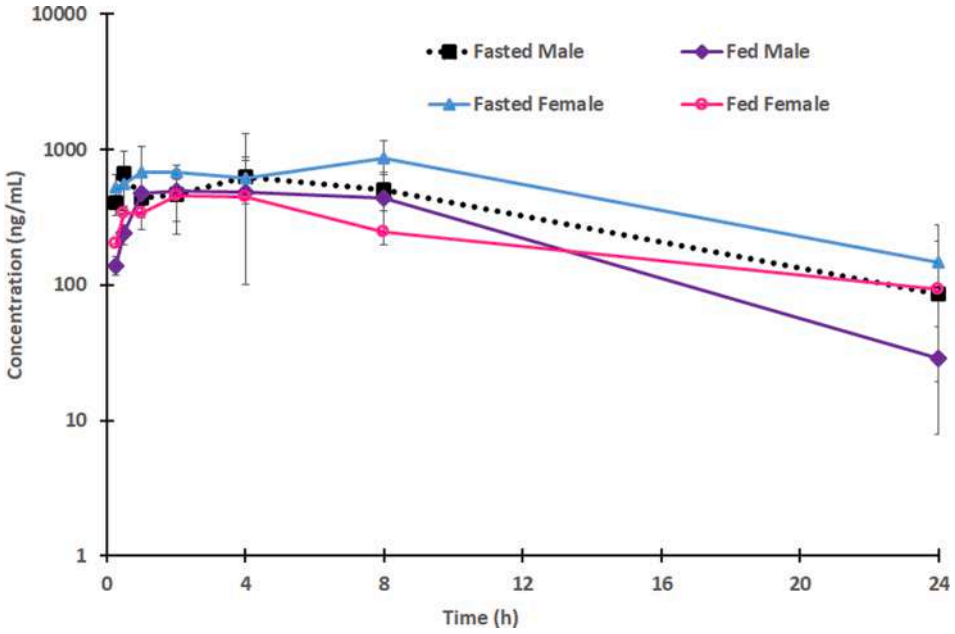

Fig. 4. Concentration-time profiles of atenolol after peroral cassette administration to male and female *Sprague Dawley* rats in fasting and fed condition at 10 mg/kg. The data presented are mean with SD error bars (n = 3) and pharmacokinetic parameters analyzed by the Mann-Whitney *U* test.

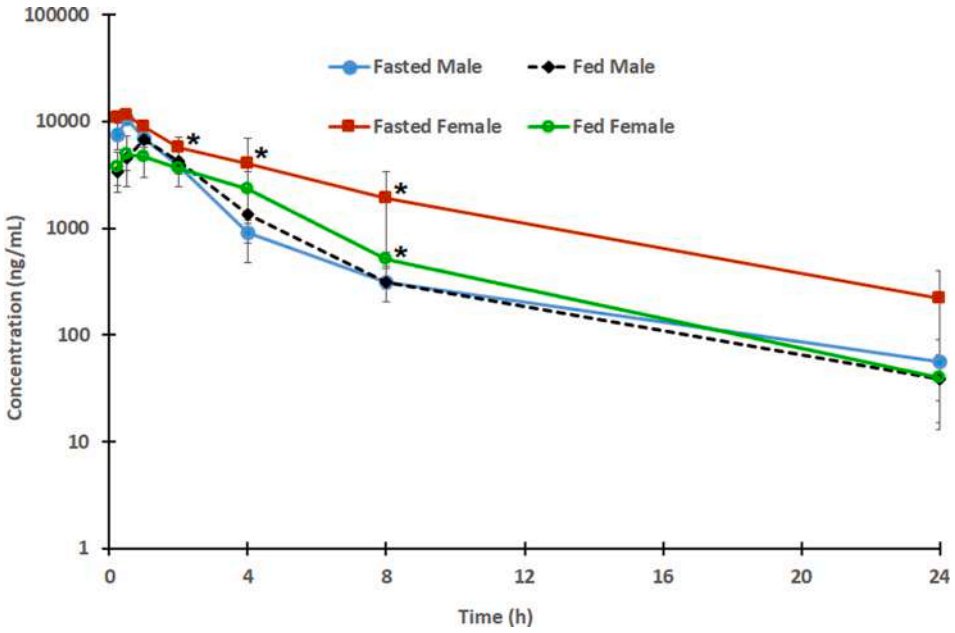

Fig. 5. Concentration-time profiles of acetazolamide after peroral cassette administration to male and female *Sprague Dawley* rats in fasting and fed condition at 10 mg/kg. The data presented are mean with SD error bars (n = 3) and pharmacokinetic parameters analyzed by the Mann-Whitney *U* test. *Statistically significant ($p < 0.05$) from male group.

influence of sex on oral and intravenous administration was compared for all four drugs tested. Administration of propranolol or atenolol by intravenous route did not show any marked differences in male or female rats in the V_d, Cl, AUC, and MRT. In contrast, diclofenac and acetazolamide showed variation in the AUC between males and females. On the other hand, propranolol showed a sex effect on oral bioavailability as females showed higher values than their male counterparts. This observation is in agreement with earlier data (Ueno and Sato, 2012) wherein it was reported that the higher oral bioavailability of propranolol in females than men indicated by higher AUC and C_{max} and a lower rate of Cl and V_d. Food intake has also influenced the oral bioavailability (decreased in fed condition) of propranolol in females, while it was comparable in males. This observation is consistent with an earlier study reported (Liedholm et al., 1990). The authors suggest that the decrease in propranolol bioavailability in females in fed conditions could be explained by reduced hepatic extraction of propranolol, probably consequent to an increased hepatic entry rate (Liedholm et al., 1990). It was further described that the extent of food effect is dependent on the degree of oral clearance of propranolol; hence is subject to high inter-individual variation and the effect on bioavailability remains to be established.

The data observed in this study proved that there is a difference in pharmacokinetics between males to females for drugs evaluated. Therefore, it is better to include both males and females in early

Table 3
Pharmacokinetic parameters of propranolol, diclofenac, atenolol, and acetazolamide after cassette oral administration in fasted *Sprague Dawley* rats (n = 3) at 10 mg/kg.

Drug	Propranolol- BCS (Class-I)		Diclofenac- BCS (Class-II)		Atenolol- BCS (Class-III)		Acetazolamide- BCS (Class-IV)	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
C_{max} (ng/mL)	747.89 (229.61–1044.87)	2370.18 (1152.50–2385.21)*	18963.68 (10593.70–22042.06)	19262.63 (18882.24–28954.28)	619.53 (557.20–1406.19)	879.13 (755.56–1148.06)	11269.20 (9067.53–11310.18)	11930.27 (11407.52–12696.07)
T_{max} (hr)	0.25 (0.25–0.5)	0.25 (0.25–0.5)	0.25 (0.25–0.5)	0.25 (0.25–0.5)	8 (4–8)	8 (2–8)	0.5 (0.25–0.5)	0.5 (0.25–0.5)
$T_{1/2}$ (hr)	1.46 (1.33–6.18)	4.26 (1.04–4.33)	2.47 (2.42–12.63)	8.65 (6.23–10.43)	3.36 (3.36–3.36)	5.38 (5.38–5.38)	5.73 (3.69–6.82)	4.88 (4.29–4.94)
K_{el} (h^{-1})	0.48 (0.112–0.52)	0.16 (0.16–0.67)	0.28 (0.06–0.29)	0.08 (0.07–0.11)	0.21 (0.16–0.29)	0.13 (0.08–0.19)	0.12 (0.10–0.19)	0.14 (0.14–0.16)
$AUC_{0\text{-last}}$ (ng.h/mL)	871.19 (229.76–1049.21)	2594.77 (1046.43–4002.07)*	18097.88 (10950.63–18930.16)	36168.05 (34028.91–78173.04)*	8098.33 (4936.13–9388.77)	11871.80 (7004.50–16553.89)	21729.33 (20059.50–22233.81)	45170.78 (28202.47–75861.83)*
$AUC_{0\text{-inf}}$ (ng.h/mL)	884.37 (233.08–1061.26)	2603.24 (1049.01–4022.53)*	18104.75 (13367.82–18943.98)	41933.69 (36723.29–85885.53)*	8187.77 (4024.14–10521.33)	7301.36 (4854.62–9125.37)	22246.31 (20906.91–22349.74)	46507.20 (28532.53–78788.87)*
$MRT_{0\text{-last}}$ (h)	1.25 (1.19–1.94)	1.43 (1.01–2.02)	2.57 (1.89–4.93)	6.15 (5.73–6.88)*	7.55 (5.77–11.39)	9.03 (7.93–10.36)	3.29 (2.77–3.52)	5.58 (3.25–6.33)
Vz_ obs (L/kg)	82.44 (29.82–100.76)	15.26 (14.28–24.02)*	1.93 (1.88–13.64)	2.45 (1.45–3.59)	5.92 (3.20–8.87)	10.64 (7.21–14.08)	3.72 (2.38–4.70)	1.51 (0.91–2.17)*
Cl_obs (mL/min/kg)	188.46 (157.05–715.06)	64.02 (41.43–148.88)*	9.21 (8.80–12.47)	3.97 (1.94–4.54)*	20.36 (16.26–24.34)	22.83 (19.61–25.37)	7.49 (7.46–7.97)	3.58 (2.12–5.84)*
%F	43.39 (11.44–52.26)	122.61 (59.45–189.12)*	137.35 (83.11–143.67)	112.14 (105.50–242.37)	94.28 (57.47–109.31)	123.57 (72.90–172.30)	115.98 (107.06–118.67)	165.96 (103.61–278.72)

All the values are mentioned as Median (Range) and analyzed by the Mann-Whitney *U* test. C_{max}: maximum concentration, T_{max}: time at which maximum concentration was observed, $AUC_{0\text{- last}}$: area under the curve for all blood time points. $AUC_{0\text{- inf}}$: area under the curve from time zero extrapolated to infinite time, MRT: mean residence time. Vz_ obs: volume of distribution based on the terminal phase. Cl_obs: clearance based on the terminal phase. %F: bioavailability. *Statistically significant ($p < 0.05$) from the male group.

Table 4
Pharmacokinetic parameters of propranolol, diclofenac, atenolol, and acetazolamide after cassette oral administration in fed *Sprague Dawley* rats (n = 3) at 10 mg/kg.

Drug	Propranolol- BCS (Class-I)		Diclofenac- BCS (Class-II)		Atenolol- BCS (Class-III)		Acetazolamide- BCS (Class-IV)	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
C_{max} (ng/mL)	523.706 (224.04–707.46)	438.92 (198.17–1548.80)	6484.36 (4027.46–8336.56)	5602.55 (4009.3–10967.15)	382.73 (374.49–931.92)	602.85 (438.46–744.89)	7143.89 (4907.35–8138.70)	6047.25 (3699.08–6559.84)
T_{max} (hr)	0.5 (0.25–1)	0.5 (0.5–2)	0.5 (0.25–1)	0.25 (0.25–0.5)	2 (2–4)	4 (2–4)	1 (0.5–1)	0.5 (0.5–2)
$T_{1/2}$ (hr)	1.38 (1.03–1.38)	3.23 (1.56–4.68)*	3.73 (2.11–9.09)	3.25 (3.19–4.85)	4.49 (3.38–6.30)	5.92 (5.14–23.35)	3.75 (3.07–5.81)	3.78 (2.98–4.16)
K_{el} (h^{-1})	0.50 (0.50–0.67)	0.21 (0.15–0.44)*	0.19 (0.08–0.33)	0.21 (0.14–0.22)	0.15 (0.11–0.20)	0.12 (0.03–0.13)	0.18 (0.12–0.23)	0.18 (0.17–0.23)
$AUC_{0\text{-last}}$ (ng.h/mL)	769.69 (315.49–1144.39)	1414.82 (577.41–1984.90)	18610.73 (10512.14–22033.86)	28573.34 (22166.95–34765.64)*	4000.41 (3810.82–9955.72)	4837.76 (4469.93–6439.79)	18985.17 (15004.43–24279.08)	20270.18 (19274.95–24648.36)
$AUC_{0\text{-inf}}$ (ng.h/mL)	796.72 (319.50–1151.61)	1420.70 (588.98–1995.42)	18616.24 (11646.90–23049.73)	28728.57 (23120.65–35021.46)*	4280.65 (3844.19–10266.03)	5104.73 (4724.61–13698.10)	19087.45 (15563.15–24407.91)	20683.22 (19420.61–24754.82)
$MRT_{0\text{-last}}$ (h)	1.50 (1.27–1.65)	2.66 (1.88–3.14)*	4.12 (2.83–5.83)	5.33 (4.54–5.65)	7.71 (6.66–8.24)	7.55 (6.76–11.20)	3.26 (2.78–4.75)	4.0 (3.57–4.86)
Vz_ obs (L/kg)	25.05 (12.91–62.69)	33.83 (32.79–38.23)	2.33 (1.63–11.26)	1.63 (1.31–3.03)	12.69 (6.31–21.26)	18.09 (14.52–24.59)	2.84 (1.81–5.38)	2.81 (1.74–2.90)
Cl_obs (mL/min/kg)	209.19 (144.72–521.65)	117.31 (83.52–282.97)	8.95 (7.23–14.31)	5.80 (4.76–7.21)*	38.93 (16.23–43.36)	32.65 (12.17–35.28)	8.73 (6.83–10.71)	8.06 (6.73–8.58)
%F	38.34 (15.71–57.00)	66.85 (27.29–93.80)	141.24 (79.78–173.29)	88.59 (68.73–107.79)	46.57 (44.37–115.91)	50.35 (46.52–67.03)	101.33 (80.08–129.58)	74.47 (70.82–90.56)

All the values are mentioned as Median (Range) and analyzed by the Mann-Whitney *U* test. C_{max}: maximum concentration, T_{max}: time at which maximum concentration was observed, $AUC_{0\text{- last}}$: area under the curve for all blood time points. $AUC_{0\text{- inf}}$: area under the curve from time zero extrapolated to infinite time, MRT: mean residence time. Vz_ obs: volume of distribution based on the terminal phase. Cl_obs: clearance based on the terminal phase. %F: bioavailability. *Statistically significant ($p < 0.05$) from the male group.

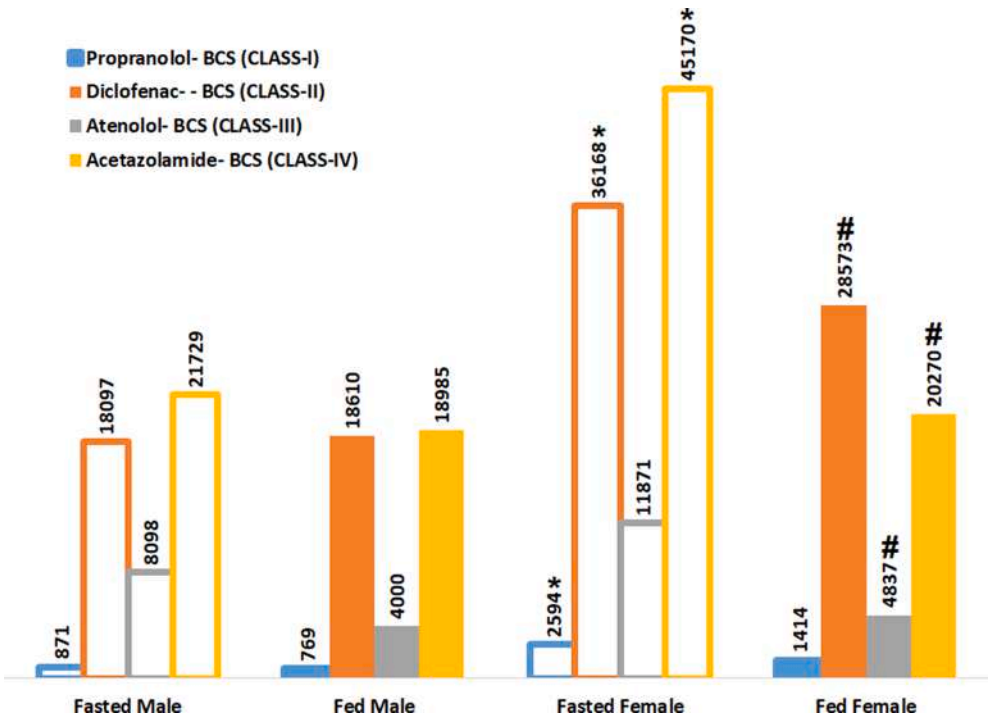

Fig. 6. The area under the curve (AUC) for propranolol, diclofenac, atenolol, and acetazolamide after peroral cassette administration to male and female *Sprague Dawley* rats in fasting and fed condition at 10 mg/kg. The data presented are median (n = 3) and pharmacokinetic parameters analyzed by the Mann-Whitney *U* test. *Statistically significant ($p < 0.05$) from fasted male group. #Statistically significant ($p < 0.05$) from fed male group.

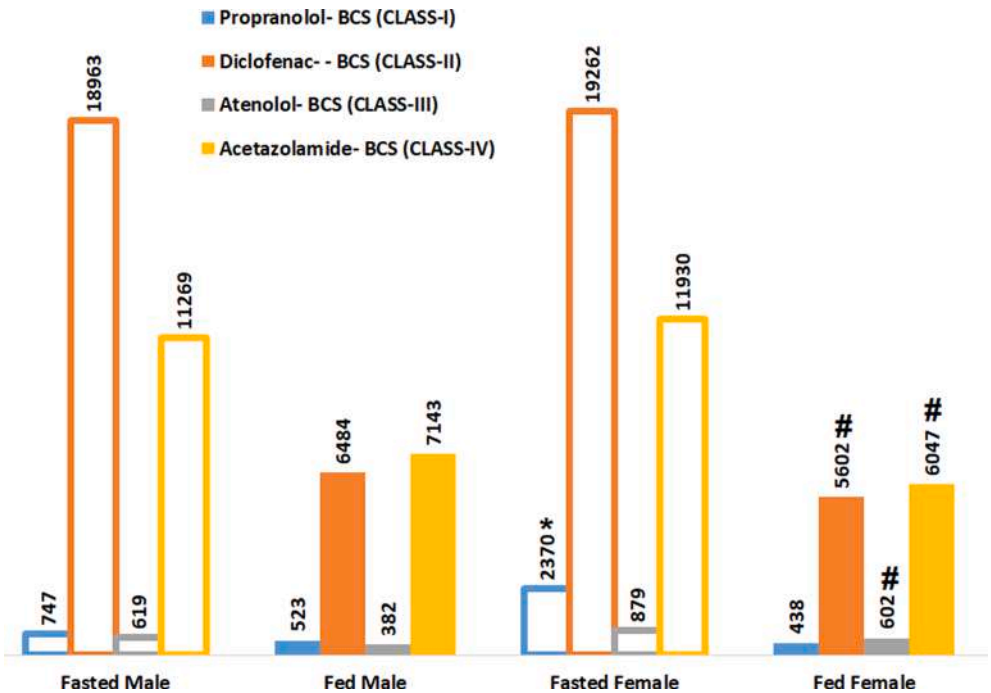

Fig. 7. Maximum concentration (C_{max}) for propranolol, diclofenac, atenolol, and acetazolamide after peroral cassette administration to male and female *Sprague Dawley* rats in fasting and fed condition at 10 mg/kg. The data presented are median (n = 3) and pharmacokinetic parameters analyzed by the Mann-Whitney *U* test. *Statistically significant ($p < 0.05$) from fasted male group. #Statistically significant ($p < 0.05$) from fed male group.

preclinical drug discovery as per 3Rs. Thus, for exploratory studies, investigation can be carried out with sex of choice, which would help to extrapolate the dose selection based on sexual difference applying statistical power in future investigations. Frequently, the dose is calculated mainly based on age, body weight, and body surface area. Due to the impact of gender differences on metabolism, sex should be also considered while prescribing the drugs in the future. Phase I metabolism enzyme, CYP3A4, is responsible for the metabolism of over 30% of therapeutic drugs, which exhibits higher oxidizing activity in women than in men (van Dyk et al., 2018). The drugs like propranolol and diclofenac used in this study are also metabolized by CYPs (propranolol - CYP1A2 and diclofenac - CYP2B6 and CYP2C9). Nonetheless, some

researchers have reported the absence of a sex difference in drug metabolism. The activity of several other CYP (CYP2C19, CYP2D6, CYP2E1) isozymes and the conjugation (glucuronidation) activity involved in drug metabolism may be higher in men than in women (Tanaka, 1999). Ten drug-metabolizing enzymes and transporters genes were found to be differentially expressed with more than 1.5-fold differences, including SLC3A1, CYP7A1, ACSL4, CYP3A7, GSTA1, CYP3A4, GSTA2, UGT2B17, SLC13A1, and ADH1A (Yang et al., 2012). Similarly, the sex differences in rats could lead to variation in drug metabolism. The sexual dimorphism in rat drug metabolism is due to the differential expression of various sex-dependent forms of P450. In some cases, the P450 is sex-specific; P450 2C11 (CYP2C11) is expressed only in male hepatocytes, whereas P450 2C12 is limited to the female liver (Shapiro et al., 1995).

The simultaneous intake of food and drugs can have a strong impact on drug release, absorption, distribution, metabolism, and/or elimination (Koziolek et al., 2019). Food's effect on the pharmacokinetics of many drugs is a well-known concept. This study depicts that by using the BCS class concept one can select the fed and fasting condition. Compounds with high solubility have less effect on fed and fasting conditions, whereas compounds with low solubility were observed with decreased C_{max} and increased MRT in fed conditions. The intake of food and/or drinks other than water can also affect the pharmacokinetic profile for different specific or unspecific reasons. The most prominent example of a specific pharmacokinetic food-drug effect is the interaction between grapefruit juice and drugs like cyclosporine and felodipine (Dahan and Altman, 2004). The interaction can result through different mechanisms, including the inhibition of CYP3A4 metabolism as well as inhibition of uptake and efflux membrane transporters (Tanaka, 1999). In fed conditions, transit time will increase for the drug mainly contributed by delayed gastric emptying and vice versa in the fasting condition. Therefore, the absorption of compounds with high solubility and high permeability are unaffected whereas compounds with low solubility and low permeability will remain in the stomach for a long time before reaching the highly absorptive region, the small intestine, for getting absorbed (Dahan and Altman, 2004). In addition, water can affect drug distribution and therefore absorption of drugs. Moreover, the existence of discontinuous water pockets within the small intestine, which can have significant implications for the rates and extents of dissolution, precipitation, and absorption of oral dosage forms (Mudie et al., 2014).

In the current study, the %F value observed was >100% in a few instances. In general, the %F value of > 100% could be possible due to the entero-hepatic recycling, where the AUC of oral increases while the AUC of intravenous decreases. Cassette data can be compared further with individual compound data for more clarity. Literature suggests that there is sufficient entero-hepatic circulation for diclofenac this could be one of some reasons for more than 100% bioavailability (Brune et al., 1987; Zhong et al., 2016). Moreover, lower clearance of acetazolamide after oral administration (observed in few groups) could be the reason for more than 100% bioavailability, though it needs further investigations to know exact reasons. On the other hand, the bioavailability of BCS Class I compounds could be compromised due to their fast absorption and first-pass metabolism. Whereas Class II and IV compounds have compromised solubility but a wide range of absorption and bioavailability can be expected. Similarly, for Class III compounds permeability is the rate-limiting step for bioavailability (Parr et al., 2016). At the same time, both uptake and efflux transporters also play a critical role in the bioavailability of various drug molecules. Hence in 2005, BDDCS was introduced to predict the drug-drug interaction at the level of the transporter (Benet, 2013).

5. Conclusion

Four compounds were selected based on BCS classification for cassette formulation preparation and the pharmacokinetics were evaluated. The observed data signifies variation in pharmacokinetics between male to female rats for drugs tested, hence the dosing can be adjusted accordingly. It was also noticed that food can majorly affect the pharmacokinetics for class II and class IV compounds. Moreover, compounds with high solubility showed minimum pharmacokinetic variability contributed by fed and fasting conditions, whereas compounds with low solubility considerably decreased C_{max} and increased MRT in fed conditions. Thus, the BCS class concept could be considered while selecting the fed and fasting condition in pharmacokinetic studies. Overall, the data in this study support the implementation of BCS classification for the selection of compounds for cassette dosing. Additional studies using various animal models and drugs from different BCS classes having dissimilar chemical structures are necessary to validate this valuable observation.

CRediT authorship contribution statement

Satish Kumar: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Surendra Yadav Ravulapalli:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Sudhir Kumar Tiwari:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Sumeet Gupta:** Data curation, Formal analysis, Writing – review & editing. **Anroop B. Nair:** Data curation, Formal analysis, Writing – review & editing. **Shery Jacob:** Data curation, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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