

## Stereospecific pharmacokinetic characterization of liquiritigenin in the rat

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### Abstract

Liquiritigenin is a chiral flavonoid present in licorice and other medicinal plants. The nature of its biological fate with respect to the individual enantiomers has not been examined. In this study, we characterize, for the first time, the stereoselective pharmacokinetics of liquiritigenin. Liquiritigenin was intravenously (20 mg/kg) and orally (50 mg/kg) administered to male Sprague-Dawley rats (n = 4 per route of administration). Concentrations in serum and urine were characterized via stereospecific reversed-phase, isocratic HPLC method with UV detection. Serum concentrations were quantified but rapidly fell to undetectable levels. S-liquiritigenin showed a short half-life (0.25-0.54 h), while a better estimation of half-life (26-77 h) and other pharmacokinetic parameters was observed using urinary data. The flavonoid is predominantly excreted via non-renal routes ( $f_e$  values of 0.16-3.46 %), and undergoes rapid and extensive phase II metabolism. Chiral differences in the chemical structure of the compound result in some pharmacokinetic differences. Serum concentrations rapidly declined, making modeling difficult. S-liquiritigenin showed an increased urinary half-life.

**Keywords:** Liquiritigenin; Pharmacokinetics; Stereospecific; Flavonoid; Chiral

### INTRODUCTION

Flavonoids are a group of polyphenolic compounds of low molecular weight (200-600 g/mol) that present a common benzo- $\gamma$ -pyrone structure (1). Among the classes of flavonoids, flavanones have been defined as citrus flavonoids due to their almost unique presence in citrus fruits (2). Furthermore, flavanones have also been reported in other plant based foods (3-5) as well as multiple nutraceuticals, natural health products, and traditional medicines in widely varying concentrations (6-11). Some flavanones present a unique structural feature known as chirality, which distinguishes them from other classes of flavonoids (12-14). Liquiritigenin belongs to the chiral flavanone family and is an important ingredient presents in liquorice (15) and some Chinese medicinal herbs (16). The achiral pharmacokinetics of liquiritigenin, and its corresponding glycosides have been reported in the literature (17). Differences in the

disposition of an individual enantiomer may result in significant differences in their health benefits or toxic effects in humans (18-27). The chiral pharmacokinetics of liquiritigenin have only been preliminarily evaluated by our laboratory and one other group. However, to our knowledge there are no studies that have comprehensively assessed the stereospecific serum and urinary pharmacokinetics and disposition of liquiritigenin and its glucuronidated metabolite in humans or rodents after intravenous and oral administration (16,28). To more thoroughly understand how liquiritigenin is absorbed, metabolized, distributed, and excreted and to be able to better understand or predict its disposition, pharmacological activity, as well as therapeutic and toxic effects, especially in light of the lipophilicity, a stereospecific HPLC method has been developed and

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validated to examine for the first time the stereoselective pharmacokinetics of liquiritigenin in rat serum and urine after intravenous and oral administration.

## MATERIALS AND METHODS

### Materials

Trans-stilbene, 7 - ethoxycoumarin,  $\beta$  - glucuronidase from *Escherichia coli* type IX-A,  $\beta$  -glucuronidase from *Helix pomatia* type HP-2, and halothane were purchased from Sigma Aldrich (USA). Racemic liquiritigenin was purchased from Extrasynthese (France). HPLC grade acetonitrile and water were purchased from J. T. Baker (USA). Phosphoric acid was purchased from Aldrich Chemical Co. Inc. (USA). Silastic<sup>®</sup> laboratory tubing was purchased from Dow Corning Corporation (USA). Intramedic<sup>®</sup> polyethylene tubing was purchased from Becton Dickinson Primary Care Diagnostics, Becton Dickinson and Company (USA). Monoject<sup>®</sup> 23 gauge (0.6 mm  $\times$  25 mm) polypropylene hub hypodermic needles were purchased from Sherwood Medical (USA). Synthetic absorbable surgical sutures were purchased from Wilburn Medical US (USA). Rats were obtained from Simonsen Labs (USA). Ethics approval for animal experiments was obtained from University of Manitoba.

### Methods

#### Animals and surgical procedures

Male Sprague-Dawley rats (200-240 g) were obtained from Simonsen Labs (USA) and given food (Purina Rat Chow 5001) and water *ad libitum* in the animal facility for at least 5 days before use. Rats were housed in temperature-controlled rooms with a 12 h light/dark cycle. The day before the pharmacokinetic experiment the right jugular veins of the rats were catheterized with sterile silastic cannula (Dow Corning, USA) under halothane anesthesia. This involved exposure of the vessel prior to cannula insertion. After cannulation, the Intramedic PE-50 polyethylene tubing (Becton, Dickinson and Company, USA) connected to the cannula was exteriorized through the dorsal skin. The cannula was flushed with 0.9% saline. The

animals were transferred to metabolic cages and were fasted overnight. Animal ethics approval was obtained from University of Manitoba Office of Research Ethics and Compliance.

#### Pharmacokinetic study

Eight male Sprague Dawley rats (average weight: 250 g) were cannulated as described in the previous section. Each of the animals were placed in separate metabolic cages, allowed to recover overnight, and fasted for 12 h before dosing. On the day of experiment, the animals were dosed either intravenously or orally with or racemic liquiritigenin (20 mg/kg IV, 50 mg/kg PO) dissolved in 2% DMSO and 98% PEG-600 (n = 4 for each treatment group). Animals received water *ad libitum* pre- and post-dosing, and food (Purina Rat Chow 5001) was provided 2 h post-dosing. Doses were selected based on previous use in similar pharmacokinetic studies and sensitivity of analytical instrumentation. Serial blood samples (0.30 mL) were collected at 0, 1 min, 15 min, and 30 min, then 1, 2, 4, 6, 12, 24, 48, and 72 h after intravenous administration or at 0, 15 min, 30 min, then 1, 2, 4, 6, 12, 24, 48, and 72 h after oral administration. At 72 h after administration, the animals were euthanized and exsanguinated. Immediately after all the blood collection time points (except the terminal point); the cannula was flushed with the same volume of 0.9% saline to replenish the collected blood volume. The samples were collected into regular polypropylene microcentrifuge tubes, centrifuged at 5,000 RPM for 5 min (Beckman Microfuge centrifuge, Beckman Coulter Inc., USA), and the serum was collected.

The serum was divided into two equal fractions into separate regular polypropylene microcentrifuge tubes labeled as free and total serum samples and stored at  $-80$  °C until further sample preparation for HPLC analysis and validation.

Urine samples were also collected at 0, 2, 6, 12, 24, 48, and 72 h following flavonoid administration, the exact volumes were recorded and two equal aliquots were collected into separate regular polypropylene microcentrifuge tubes labeled as free and total

urine samples and stored at  $-80\text{ }^{\circ}\text{C}$  until further sample preparation for HPLC analysis and validation.

#### *Serum and urine sample preparation for analysis*

Serum and urine samples were run in duplicate with or without the addition of  $40\text{ }\mu\text{L}$  of  $500\text{ U/mL}$   $\beta$ -glucuronidase from *Escherichia coli* type IX-A and incubated in a shaking water bath at  $37\text{ }^{\circ}\text{C}$  for 2 h to liberate any glucuronide conjugates without decomposition of the parent compound. The proteins present in the serum samples (as well as the enzyme in the total samples) were precipitated using 1 mL of cold HPLC-grade acetonitrile, vortexed for 1 min (Vortex Genie-2, VWR Scientific, USA), and centrifuged at 15000 rpm for 5 min, the supernatant was transferred to new labeled 2 mL centrifuge tubes. The samples were evaporated to dryness under a constant flow of compressed nitrogen gas (29). The residue was reconstituted with  $200\text{ }\mu\text{L}$  of mobile phase, vortexed for 1 min and centrifuged at 5000 rpm for 5 min, the supernatant was transferred to HPLC vials and  $20\text{ }\mu\text{L}$  was injected into the HPLC system, the remaining volume was utilized for method validation.  $\beta$ -glucuronidase from *Escherichia coli* type IX-A cleaves specifically any glucuronidated metabolites back to the corresponding aglycones (liquiritigenin). Therefore, the samples without enzymatic hydrolysis (free samples) were utilized to determine the concentration of the aglycones, whereas the samples with enzymatic hydrolysis (total samples) were utilized to determine the concentration of the aglycones originally present plus the concentration of the major glucuronidated metabolites converted to their respective aglycones by the cleavage action of the enzyme. Finally, by subtracting the free sample concentration from the total sample, the stereospecific concentration of the glucuronidated metabolites can be calculated.

#### *Pharmacokinetic analysis*

Pharmacokinetic analysis was performed using data from individual rats for which the mean and standard error of the mean (SEM) were calculated for each group. The

elimination rate constant ( $k_{el}$ ) was estimated by linear regression of the serum concentrations in the log-linear terminal phase. In order to estimate the serum concentrations ( $C_0$ ) immediately after IV dosing, a two-compartmental model was fitted to the serum concentration versus time data using Phoenix<sup>®</sup>WinNonlin<sup>®</sup> software (V. 6.3) (Pharsight Corporation, CA). The estimated  $C_0$  was then used with the actual measured serum concentrations to determine the area under the serum concentration-time curve (AUC). The  $\text{AUC}_{0-\infty}$  was calculated using the combined log-linear trapezoidal rule for data from time of dosing to the last measured concentration ( $\text{AUC}_{0-t}$ ), plus the quotient of the last measured concentration divided by  $k_{el}$ . Non-compartmental pharmacokinetic methods were used to calculate the different pharmacokinetic parameters in the terminal phase, namely total clearance ( $\text{CL}_{tot}$ , by dividing dose by  $\text{AUC}_{0-\infty}$ ) and volume of distribution ( $V_{ss}$ , by multiplying dose by the  $\text{AUMC}_{0-\infty}$  and dividing it by the square of  $\text{AUC}_{0-\infty}$ ). Based on the cumulative urinary excretion, the fraction excreted unchanged in urine ( $f_e$ , by dividing the total cumulative amount of flavonoid excreted in urine ( $\sum X_u^{\infty}$ ) by the dose), renal clearance ( $\text{CL}_{renal}$ , by multiplying  $f_e$  by  $\text{CL}_{tot}$ ), hepatic clearance ( $\text{CL}_{hepatic}$ , by subtracting  $\text{CL}_{renal}$  from  $\text{CL}_{tot}$ , assuming that hepatic clearance is equivalent to non-renal clearance).

In order to assess the pharmacokinetic parameters from urinary data, the urinary elimination rate constant ( $k_e$ ) and half-life were first characterized employing non-compartmental pharmacokinetic methods using Phoenix<sup>®</sup>WinNonlin<sup>®</sup> software (V. 6.3) (Pharsight Corporation, CA). The other pharmacokinetic parameters were calculated as described above but instead of employing serum elimination rate constant ( $k_{el}$ ), urine elimination rate constant ( $k_e$ ) was utilized.

#### *Statistical analysis*

Compiled data were presented as mean and standard error of the mean (mean  $\pm$  SEM). Where possible, the data were analyzed for statistical significance using Excel software. Student's t-test was employed for unpaired samples with a value of  $P < 0.05$  being considered statistically significant.

## RESULTS

*Stereospecific pharmacokinetics of intravenous liquiritigenin*

The analytical HPLC method described elsewhere was applied to the stereospecific determination of liquiritigenin (16). Linearity in the standard curves was demonstrated (0.995 for S-liquiritigenin ( $y = 0.0239x - 0.0428$ ) and 0.994 for R-liquiritigenin ( $y = 0.0101x - 0.0097$ ) in the samples for the chiral flavonoids over the concentration range studied (0.05, 0.1, 0.5, 1, 5, 10, 50, and 100  $\mu\text{g/mL}$ ; LOQ: 0.5  $\mu\text{g/mL}$ ), and chromatograms were free of interference from endogenous components. Total samples (incubated with  $\beta$ -glucuronidase from *Escherichia coli* Type IX-A) demonstrated the presence of at least one glucuronidated metabolite based on the increase in the aglycone parent compound (liquiritigenin) concentrations after the enzymatic hydrolysis, which was assessed as described previously.

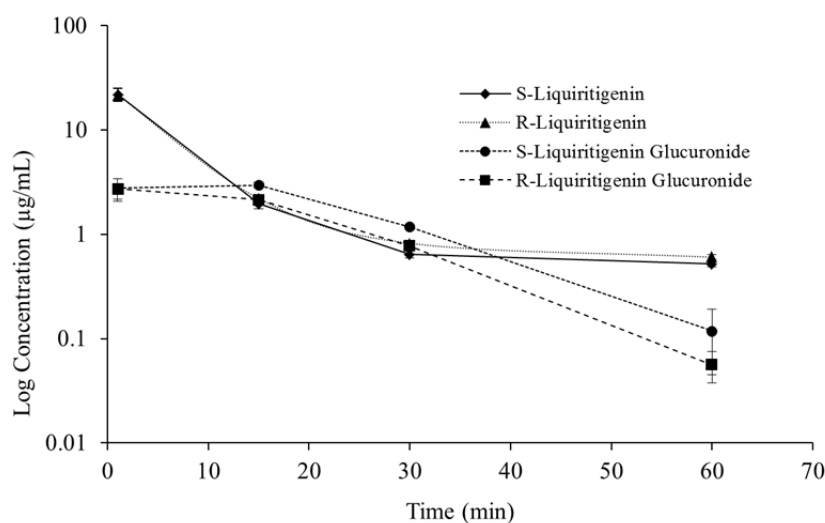
The serum disposition profiles observed for liquiritigenin demonstrated some differences in stereoselective disposition. Independent of the enantiomeric form, the flavonoid was characterized by a rapid decline in concentrations detectable (Fig. 1). The intercept of the concentration-time profile of liquiritigenin suggests that the compound distributes beyond a central vascular compartment (Fig. 1). The elimination phase for the parent compound was characterized with half-lives between 0.2-0.5 h.

S-liquiritigenin exhibited a shorter  $t_{1/2}$  ( $0.253 \pm 0.712$  h) than its counterpart ( $0.543 \pm 0.071$  h) in serum (Table 1).

The glucuronidated metabolites of liquiritigenin enantiomers exhibited similar concentration-time profiles with no indication of enterohepatic recycling observed (Fig. 1).

Non-compartmental analysis of the serum concentrations indicated several differential pharmacokinetic parameters of liquiritigenin enantiomers (Table 1). For instance, R-liquiritigenin showed significantly higher values for volume of distribution ( $V_{ss}$ ) while, fraction excreted unchanged in the urine ( $f_e$ ), and serum elimination rate constant ( $k_{el}$ ) had larger values for the S-enantiomer.

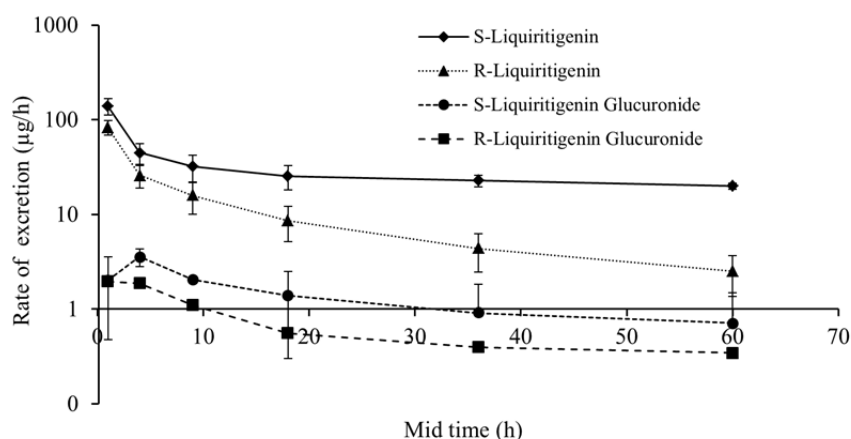
The differences in certain pharmacokinetic parameters between liquiritigenin enantiomers demonstrate that this flavonoid may be stereospecifically metabolized. For instance, the significantly higher  $V_{ss}$  and lower values of  $f_e$  and serum  $k_{el}$  of R-liquiritigenin than S-liquiritigenin is consistent with R-liquiritigenin residing longer in the body with a correspondingly higher serum half-life. The increased  $f_e$  of S-liquiritigenin ( $3.460 \pm 0.143\%$ ) compared to that of R-liquiritigenin ( $0.157 \pm 0.131\%$ ) denotes that a much larger percentage of S-liquiritigenin is excreted unchanged in the urine compared to its counterpart. These differences may be partially attributed to the observed deeper penetration into tissues (higher  $V_{ss}$ ) of R-liquiritigenin.



**Fig. 1.** Serum concentration-time profiles of liquiritigenin enantiomers following intravenous administration of racemic liquiritigenin (20 mg/kg) in rats ( $n = 4$ ). Data are presented as Mean  $\pm$  SEM.

**Table 1.** Stereospecific pharmacokinetics of liquiritigenin in serum after IV administration in rats (20 mg/kg) (Mean  $\pm$  SEM, n = 4). (a) Denotes statistical significant difference ( $P < 0.05$ ) between enantiomers.

Pharmacokinetic parameter	S-liquiritigenin	R-liquiritigenin
AUC <sub>0-∞</sub> (μg.h/mL)	4.670 $\pm$ 0.591	4.226 $\pm$ 0.278
V <sub>ss</sub> (L/kg)	2.675 $\pm$ 0.724	4.637 $\pm$ 0.344 <sup>a</sup>
CL <sub>renal</sub> (L/h/kg)	0.758 $\pm$ 0.241	0.010 $\pm$ 0.001 <sup>a</sup>
CL <sub>hepatic</sub> (L/h/kg)	2.344 $\pm$ 1.347	2.655 $\pm$ 0.318
CL <sub>total</sub> (L/h/kg)	3.033 $\pm$ 1.126	2.665 $\pm$ 0.708
f <sub>e</sub> (%)	3.460 $\pm$ 0.143	0.157 $\pm$ 0.131
k <sub>el</sub> (h <sup>-1</sup> ) serum	3.434 $\pm$ 0.811	1.227 $\pm$ 0.506 <sup>a</sup>
k <sub>el</sub> (h <sup>-1</sup> ) urine	0.009 $\pm$ 0.005	0.026 $\pm$ 0.022
t <sub>1/2</sub> (h) serum	0.253 $\pm$ 0.172	0.531 $\pm$ 0.071
t <sub>1/2</sub> (h) urine	77.03 $\pm$ 1.213	26.714 $\pm$ 1.762 <sup>a</sup>

**Fig. 2.** Rate of urinary excretion of free and glucuronidated liquiritigenin enantiomers following intravenous administration of racemic liquiritigenin (20 mg/kg) in rats (n = 4). Data are presented as Mean  $\pm$  SEM.

It was also observed that liquiritigenin enantiomers had variable degrees of hepatic clearance. Assuming that hepatic clearance is equivalent to non-renal clearance, R-liquiritigenin is predominately cleared via hepatic elimination (fraction excreted in urine, f<sub>e</sub> of 0.157  $\pm$  0.131) while S-liquiritigenin showed a lower degree of hepatic clearance with an f<sub>e</sub> value of 3.460  $\pm$  0.143% indicating some clearance by the liver (Table 1).

The glucuronidated metabolites of liquiritigenin previously identified in serum were also detected in the urine samples (Fig. 2).

The observed terminal urine half-life of liquiritigenin was not significantly different between enantiomers. However, it was clearly observed that the urine half-life was significantly higher than the plasma half-life for both enantiomers. The observed serum half-lives for S- and R-liquiritigenin were 0.253  $\pm$  0.172 h and 0.5431  $\pm$  0.071 h respectively, while the urinary half-lives were

77.031  $\pm$  1.213 h and 26.714  $\pm$  1.762 h for S- and R-liquiritigenin, respectively (Table 1).

The pharmacokinetic parameters derived from urine were calculated (Table 1). It can be observed that employing serum significantly underestimates AUC<sub>0-∞</sub>, and half-life, while k<sub>el</sub> was overestimated. Thus, urine may provide more significant pharmacokinetic parameters indicating that liquiritigenin enantiomers have long half-lives (up to 77 h) and high volumes of distribution suggesting they distribute in the body.

In the rat liquiritigenin is rapidly being metabolized to different glucuronides, these are likely being excreted in the bile and feces. Despite the differences in solubility and lipophilicity nature of these compounds (parent and glucuronidates compounds) it can be observed that they have similar rates of excretion (Fig. 2). This indicates that both parent drug and metabolite undergo similar magnitude of apparent elimination (since their

elimination phases are parallel) indicating that the glucuronide conjugates are formation-rate limited and that their half-lives would be a reflection of the elimination of the parent flavonoids.

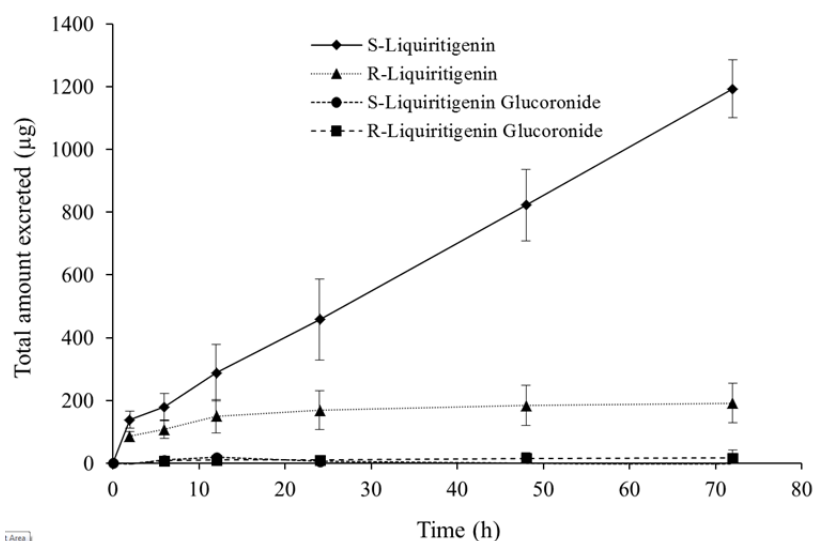
The total cumulative urinary excretion plot (Fig. 3) indicates that the two enantiomers are excreted in the urine unchanged (parent compounds) to variable extents. The total amount of S-liquiritigenin excreted unchanged was higher than R-liquiritigenin. This is consistent with the higher  $f_e$  value of  $3.460 \pm 0.143$  of the S-enantiomer compared to the R-enantiomer ( $f_e$  value of  $0.157 \pm 0.131$ ).

### Stereospecific pharmacokinetics of oral liquiritigenin

Following oral administration of liquiritigenin (50 mg/kg), the serum concentration vs. time curves indicated low absorption of free flavonoid (Fig. 4) (Table 2). Liquiritigenin enantiomers were glucuronidated but to variable degrees. The

conjugated metabolite was detected out to 6 h. The method was unable to capture a complete picture of disposition for free S- and R-liquiritigenin. For this reason, non-compartmental modeling using WinNonlin<sup>®</sup> was employed to determine the pharmacokinetic parameters of total S- and R-liquiritigenin following oral dosing (Table 2). Significant differences between enantiomers following oral administration were apparent. Bioavailability (F) was calculated by dividing the AUC of oral administration by the AUC of IV administration with dose adjustment (Table 2).

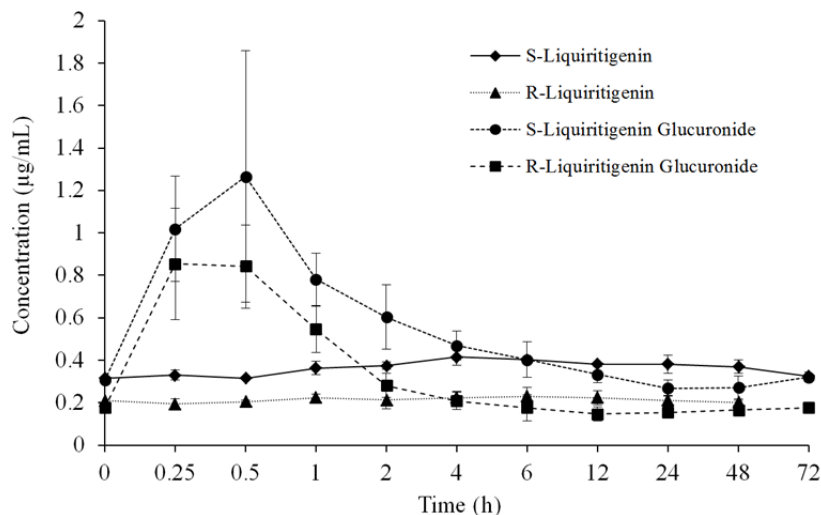
Total R-liquiritigenin had a greater calculated bioavailability compared to total S-liquiritigenin (~ 16% vs ~ 5% respectively). The lower bioavailability of one enantiomeric form over the other may suggest an extensive stereoselective first-pass metabolism. This is supported by the larger amount of R-liquiritigenin glucuronides seen following enzyme incubation.



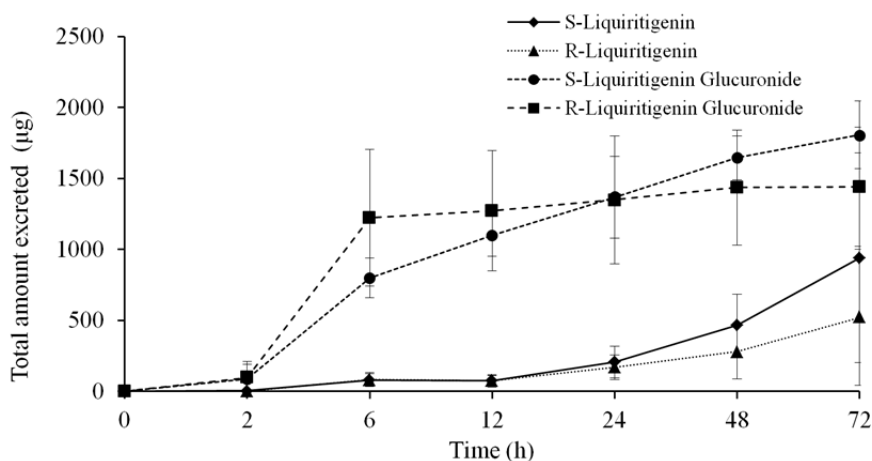
**Fig. 3.** Cumulative free and glucuronidated liquiritigenin enantiomers excreted in urine following intravenous administration of racemic liquiritigenin (20 mg/kg) in rats ( $n = 4$ ). Data are presented as Mean  $\pm$  SEM.

**Table 2.** Stereospecific pharmacokinetics of total liquiritigenin in serum after PO administration of 50 mg/kg dose in rats (Mean  $\pm$  SEM,  $n = 4$ ). (a) Denotes statistical significant difference ( $P < 0.05$ ) between enantiomers.

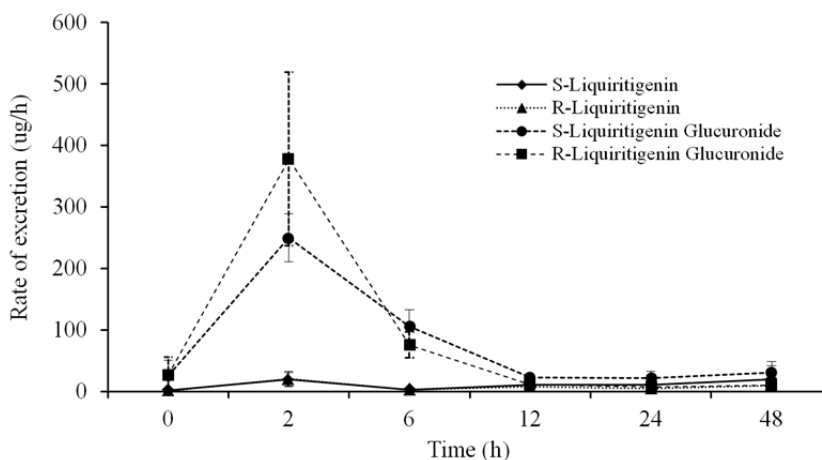
Pharmacokinetic parameter	S-liquiritigenin	R-liquiritigenin
AUC <sub>0-∞</sub> (µg.h/mL)	192.5 $\pm$ 39.07	92.53 $\pm$ 13.27 <sup>a</sup>
V <sub>ss</sub> /F (L/kg)	0.162 $\pm$ 1.012	0.868 $\pm$ 1.123
CL <sub>total</sub> /F (L/h/kg)	0.700 $\pm$ 0.851	1.450 $\pm$ 0.084 <sup>a</sup>
F (%)	16.49 $\pm$ 6.352	5.344 $\pm$ 4.792
t <sub>1/2</sub> (h) serum	4.183 $\pm$ 14.54	6.257 $\pm$ 20.43



**Fig. 4.** Serum concentration-time profiles of liquiritigenin enantiomers following oral administration of racemic liquiritigenin (50 mg/kg) in rats (n = 4). Data are presented as Mean ± SEM.



**Fig. 5.** Cumulative free and glucuronidated liquiritigenin enantiomers excreted in urine following oral administration of racemic liquiritigenin (50 mg/kg) in rats (n = 4). Data are presented as Mean ± SEM.



**Fig. 6.** Rate of urinary excretion of free and glucuronidated liquiritigenin enantiomers excreted in urine following oral administration of racemic liquiritigenin (50 mg/kg) in rats (n = 4). Data are presented as Mean ± SEM.

The values for  $V_d/F$  and  $CL/F$  were also calculated for total liquiritigenin following oral administration. These parameters vary from those calculated from IV studies. This is expected as these parameters are reflective of bioavailability. The parameters from IV data will provide the purest pharmacokinetic estimates as they are not contaminated by bioavailability, however, the incomplete disposition obtained due to poor detection renders these parameters problematic. The half-lives of liquiritigenin enantiomers did not differ significantly and ranged from 4 to 6 h (Table 2). These half-lives following oral administration were longer than those seen after IV administration (0.25-0.54 h).

Urine sample analysis following oral administration showed the presence of both the parent and glucuronide conjugate forms. The total cumulative urinary excretion plots following oral administration of liquiritigenin are reported in Fig. 5. The plots show that liquiritigenin is excreted predominantly in the glucuronidated form which parallels the results seen after IV administration of this compound.

The rate of urinary excretion plots for liquiritigenin (Fig. 6) indicates different rates of urinary excretion for each enantiomer and their corresponding glucuronidated metabolites following oral administration.

## DISCUSSION

As has been stated, pharmacokinetic characterization and analysis of liquiritigenin has been incomplete. Achiral liquiritigenin pharmacokinetics have been fairly well characterized. Many of the studies monitor the pharmacokinetics of liquiritigenin and other components of complex botanical mixtures or single plant extracts, making it difficult to know the true dose of pure liquiritigenin administered. Kang, *et al.* utilizing intravenous dosing of liquiritigenin 20 mg/kg reported approximate plasma half-lives of 5-8 minutes, clearances of 50-70 mL/min/kg,  $V_{ss}$  240-190 mL/kg, and AUC of 290-410  $\mu\text{g}\cdot\text{min}/\text{mL}$ . Data from the present study parallel these results with respect to AUC (280-385  $\mu\text{g}\cdot\text{min}/\text{mL}$ ) and clearance (30-50 mL/min/kg) but also demonstrated a comparatively greater half-life

(0.25-0.43 h) (25,26). The very short half-life of liquiritigenin reported by Kang, *et al.* was calculated utilizing plasma samples only, which may underestimate the actual half-life of liquiritigenin and also emphasizes the importance of utilizing urinary data to calculate pharmacokinetic parameters of liquiritigenin as well as similar flavonoids (25,26). Pharmacokinetic parameters of orally administered liquiritigenin given alone or in a mixture (exact dose unknown) are highly variable between existing studies with approximate plasma half-lives of 3-11 h, and AUC of 19-6594  $\mu\text{g}\cdot\text{h}/\text{L}$ . The bioavailability reported by Kang, *et al.* was 3-7% after oral doses of 20-50 mg/kg which is lower than the bioavailability reported herein (18,19,21,25-27). This discrepancy in bioavailability likely results from differences in formulation, fasting state, and vendor supplier of rats and source materials. For IV and oral formulations, Kang, *et al.* dissolved liquiritigenin in a 40:60 ratio of PEG 400:distilled water (25,26). These formulations presumably vary greatly from the PEG 600 and DMSO used in these studies which may alter the aqueous solubility and dissolution of liquiritigenin. Li, *et al.* conducted the only study to date with respect to stereospecific pharmacokinetics of liquiritigenin in urine in humans after oral administration of a botanical mixture. The authors reported fraction excreted unchanged in the urine ( $f_e$  %) of  $36.2 \pm 19.0$  S-liquiritigenin compared to  $50.9 \pm 21.6$  R-liquiritigenin (28). However, this was after a 5 g oral dose of a botanical mixture consisting of 7 herbs rather than pure liquiritigenin which may not reflect the actual pharmacokinetic profile of pure liquiritigenin. The data presented here shows significant stereoselectivity with an  $f_e$  (%) of  $3.46 \pm 0.143$  for S-liquiritigenin compared to  $0.157 \pm 0.131$  for R-liquiritigenin after intravenous administration of pure liquiritigenin in rats. It is possible that species dependent pharmacokinetics and stereoselective urinary excretion are responsible for these differences. Additionally, our results show that liquiritigenin undergoes glucuronidation upon intravenous administration, as determined by serum and urine concentrations and later



verified by treating plasma and urine samples with  $\beta$ -glucuronidase. These observations parallel findings previously mentioned. To our knowledge, this is the first literature report that has assessed the stereospecific pharmacokinetics of liquiritigenin after intravenous and oral administration of the pure racemates in both serum and urine (25,26). Previous studies except one have focused only on the racemic mixtures and utilized achiral analysis after oral ingestion (28). Our findings indicate that liquiritigenin has a relatively short half-life (0.25-0.54 h) in serum and long half-life in urine (26-77 h) after IV administration as shown previously with similar compounds (stilbenes) that belong to the same family of polyphenols (4.7 fold to 16 fold higher half-lives in urine compared to plasma half-lives). Notably, liquiritigenin could be measured for only an hour in serum in this study, while in urine it could be measured up to 70 h suggesting that serum data here may be problematic and must be interpreted with caution (30,31). The discrepancy between plasma and urine half-life in different stilbenes was attributed to assay sensitivity limits that would most likely underestimate the overall half-life of these compounds. Underestimation of plasma half-life due to assay sensitivity limits has been reported before in the case of procainamide. Nevertheless, most of the pharmacokinetic studies only collect samples up to 24 h post-dose, which could underestimate the elimination phase and pharmacokinetic parameters (32). These discrepancies suggest that the serum half-life is likely significantly underestimating the overall half-life of liquiritigenin enantiomers due to assay sensitivity limits in serum. Thus, the pharmacokinetics and biodisposition of these compounds need to be reconsidered on the basis of their chirality and glucuronidated metabolites in the fact that urine provides higher concentrations of these xenobiotics to assess these parameters particularly if serum concentrations could not be detected long enough to obtain a good elimination phase. Furthermore, the large volumes of distribution (2.7-4.6 L/kg) of liquiritigenin enantiomers are significantly larger than the total blood volume

(0.054 L/kg) and the total water volume (0.668 L/kg) in the rat indicating that both enantiomers of these compounds are exiting the blood and penetrating deeply into the tissues (33). These large volumes of distribution ( $V_{ss}$ ) values correlate with the lipophilic nature of liquiritigenin (LogP value of 2.2), which might indicate its preferential binding to tissues and preference to reside in the body. Based on the clearance values it can be observed that liquiritigenin is mainly excreted via non-renal routes (assuming that hepatic clearance is equivalent to non-renal clearance), which is also verified by their generally low fraction excreted in urine ( $f_e$ , values of 0.16-3.46% (Table 1)). Based on these pharmacokinetic data, liquiritigenin appears to be exiting the vasculature and distributing to the different tissues in the body.

## CONCLUSION

The stereospecific pharmacokinetics of intravenously and orally administered liquiritigenin in rats are reported for the first time within this study in both serum and urine. The results indicate that liquiritigenin is highly distributed and rapidly glucuronidated. It likely undergoes elimination via non-renal routes, which may indicate high achievable concentrations in the liver and the gastrointestinal tract and contribute to its poor oral bioavailability. The compound's half-life is better characterized through examining urine kinetics as it was detected in urine for up to 70 h. The chirality of liquiritigenin greatly affected its disposition in serum and urine and its overall pharmacokinetic profile. Moreover, the importance of delineating the disposition of each enantiomer and glucuronidated metabolite in urine as well as serum was shown necessary.

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