Comparative pharmacokinetics of the main compounds of Shanzhuyu extract after oral administration in normal and chronic kidney disease rats

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ABSTRACT

Ethnopharmacological relevance: Pharmacokinetic studies on traditional Chinese medicine are useful to evaluate and predict the drug efficacy and safety. The renal impairment may affect drug clearance and other pharmacokinetic processes which can increase toxicity and drug to drug interactions or cause ineffective therapy. Pharmacokinetic studies in pathological status rats might be meaningful for revealing the action mechanism and improving clinical medication of the herb medicine.

Materials and methods: A highly sensitive and rapid ultra-performance liquid chromatography–mass spectrometry (UPLC–MS) method with multiple-reaction monitoring (MRM) mode was developed and validated for simultaneous quantitation of morroniside and loganin in normal and doxorubicin-induced chronic kidney disease (CKD) rat plasma after oral administration of Shanzhuyu (fruit of Cornus officinalis) extract.

Results: Both calibration curves gave satisfactory linearity \( r^2 > 0.99 \) at linear range of 1.96–1962.5 ng mL\(^{-1}\) for morroniside, 1.53–1531.25 ng mL\(^{-1}\) for loganin. The precision and accuracy of the in vivo study were assessed by intra-day and inter-day assays. The percentages of relative standard deviation (RSD) were all within 9.58% and the accuracy (RE) was in the 6.02% to 8.11% range. The extraction recoveries of morroniside, loganin and internal standard (IS) were all 467.62% and the matrix effects ranged from 95.07% to 102.75%.

Conclusions: The pharmacokinetic behavior of morroniside and loganin in normal and CKD rat plasma was determined in this paper. The significant different pharmacokinetic parameters might partly result from the changes of P-glycoprotein and metabolic enzymes in the pathological state. The pharmacokinetic research in the pathological state might provide more useful information to guide the clinical usage of the herb medicine.

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1. Introduction

Recently, many plant-derived natural products have been used in traditional medicine for the treatment of various diseases. Cornus officinalis, a species of dogwood, has been used for thousands of years as an important folk medicine, and it is also considered as one of the 25 vegetable drugs that are most frequently applied in PR China, Japan and Republic of Korea (Cao et al., 2011; Han et al., 2014; Ma et al., 2014). Cornus officinalis exhibits a number of biological activities, including immunological regulation, reducing blood glucose, antishock, antiarrhythmia and antibiosis (Lee et al., 2011; Park et al., 2011). Pharmacological studies have demonstrated that the fruit of Cornus officinalis, called ‘Shanzhuyu’, possesses immune regulation, anti-hyperglycemia, anti-aging, anti-oxidant, renal and neural protection effects. It is widely used for treatment of kidney diseases, including diabetic nephropathy. Recently, an aqueous extract prepared from the fruit has been reported to show strong anti-proliferative activity on estrogen receptor-positive breast cancer cells (Jeong et al., 2012; Telang et al., 2012; Zhang et al., 2013).

Characterization of the pharmacokinetic properties is essential for monitoring and prediction of drug disposition in vivo because of its special significance in the evaluation of drug therapeutic effect, dose adjustment and the rational use of the drug in the clinic (Feng et al., 2013). Drugs are used to treat diseases and only
patients are the ultimate consumers. The pathological status and severity of disease seriously affects the drug absorption, which is directly related to drug efficacy and severity of side effects (Kang et al., 2014; Reid and Carlson, 2014; Schuetz et al., 2014).

Iridoid glycosides are the major active components widely distributed in Shanzhuyu (Yamabe et al., 2010; Liang et al., 2013; Zhou et al., 2013). Exploring dynamic of the iridoid glycosides may provide a helpful chemical proof for further pharmacology and active mechanism research of the herb medicine. Based on these points of view, a simple and accurate method was firstly developed and validated for simultaneous determination of the two main iridoid glycosides loganin and morroniside in normal and doxorubicin-induced chronic kidney disease (CKD) rats which were subjected to oral administration of shanzhuyu extract.

2. Experimental

2.1. Materials and reagents

Shanzhuyu raw material was purchased from Nanjing Guoyao Pharm Co. Ltd (Nanjing, China). Loganin, morroniside and chloramphenicol were purchased from Shanghai Winherb Medical S&T Development Co. Ltd (Shanghai, China). HPLC-grade acetonitrile was obtained from TEDIA Company Inc. (Fairfield, USA); formic acid and methanol were obtained from Merck KGaA (Darmstadt, Germany); Ultra-pure water was purified by an EPED super purification system (Nanjing, China). The distilled water was used for the extraction and preparation of samples. All other reagents and chemicals were of analytical grade and commercially available.

2.2. Instrument and analytical conditions

Chromatographic experiments were performed on an Acquity UPLC system (Waters Corp., Milford, MA, USA). Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm) was employed with a flow rate of 0.4 mL/min. The mobile phase consisted of 0.1% formic acid in water as solvent A and acetonitrile as solvent B. The gradient condition of the mobile phase was: B increased from 3% to 97% in 9 min. The sample injection volume was 5 μL. All separations of standards and serum samples were performed at room temperature. All sample extracts were maintained in the autosampler at 4 °C while awaiting injection.

Mass spectrometry detection was carried out using a Xevo Triple Quadrupole MS (Waters Corp., Milford, MA, USA) equipped with an electrospray ionization source (ESI) in multiple reaction monitoring (MRM) mode. Following optimization of the setting parameters, the instrument was operated in the negative mode. The parameters in the source were set as follows: capillary voltage 3.0 kV; source temperature 120 °C; desolvation gas flow 600 L/h; desolvation temperature 350 °C; cone gas flow 50 L/h. The cone voltage and collision energy were optimized for each analyte and selected values were given in Table 1. Dwell time was automatically set by MassLynx (Waters Corp., Milford, MA, USA).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Retention time (min)</th>
<th>MRM transitions</th>
<th>Cone voltage (V)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morroniside</td>
<td>4.41</td>
<td>451.0→179.0</td>
<td>18.0</td>
<td>15.9</td>
</tr>
<tr>
<td>Loganin</td>
<td>4.87</td>
<td>435.0→227.0</td>
<td>20.0</td>
<td>30.0</td>
</tr>
<tr>
<td>IS</td>
<td>6.06</td>
<td>320.8→151.9</td>
<td>20.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Table 1 MS/MS detection parameters for morroniside and loganin.

2.3. Shanzhuyu sample preparation

The shanzhuyu raw material (100 g) were extracted with 1000 mL water for 2 times and 2 h per time. The extraction solutions were combined and concentrated to 100 mL. The effective components in shanzhuyu were analyzed and evaluated before the pharmacokinetic experiment. Morroniside and loganin standards were dissolved in water at a series concentration to construct calibration curves. According to peak areas of the morroniside and loganin in the shanzhuyu extract sample, the contents of morroniside and loganin in the extract were 1.06% and 0.91%, respectively. The UPLC chromatograms of Shanzhuyu extract were displayed in the Fig.1. Peaks of all the compounds detected in UPLC-MS conditions were shown in the total ion chromatogram (Fig.1a). When the m/z of morroniside and loganin were entered into the total ion chromatogram to select their peaks, extract ion chromatogram was obtained as the Fig.1b.

2.4. Animals and induction of CKD in rats

Pathogen-free male Sprague-Dawley rats (180–220 g) were bought from the Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) and the certificate number was SCXK (Jing) 2012-0001. The animal experiment was carried out according to the Regulations of Experimental Animal Administration (State Committee of Science and Technology of the People’s Republic of China). The rats were housed in an air-conditioned animal quarter with 12 h light/12 h dark cycle at a temperature of 22 ± 2 °C and a relative humidity of 50 ± 10%.

According to previous reports, doxorubicin was dissolved in physiological saline at a concentration of 2.0 g/L. The CKD group was established by tail vein injection with 5.0 mg/kg doxorubicin for one time. After 7 days, rats with total amount of urinary protein in 24 h more than 100 mg were classified as the CKD group and used in the experiment (Chen et al., 2013; Hrenak et al., 2014).

2.5. Pharmacokinetic experiment

The rats were randomly divided normal rats oral administration shanzhuyu group and model rats oral administration shanzhuyu group (n=6). Before the test, the rats were fasted for 12 h, but were allowed water ad libitum. Then, shanzhuyu extracts were administered to the rats at a dose of 4.0 mL/kg body weight once, respectively.

The blood sample (about 0.3 mL) from the rat was drawn into a heparinized haemospasia tube at the time points of 5, 15, 30, 45,
60, 90, 120, 180, 360, 480, 720 and 1440 min separately after dosing, and then immediately centrifuged at 13,000 rpm for 10 min. The plasma samples were then transferred to Eppendorf tubes and stored at \(-80^\circ C\) until analysis.

All of the rats were put into metabolic cages for 12 h urine and fecal samples collections. The samples were collected at 12 h post-intake.

2.6. Plasma sample preparation

The plasma sample (120 μL) was precipitated with 30 μL IS and 360 μL methanol. After vortexing for 1 min, the sample was centrifuged at 3000 rpm for 10 min. Then, the organic layer was transferred to another tube, and evaporated to dryness at 35 °C in a centrifugal vacuum concentrator LABCONCO CentriVap. The residue was reconstituted in 120 μL methanol and centrifuged at 13,000 rpm for 10 min. Then, 5 μL of the supernatant was injected into the UPLC–MS system for analysis.

2.7. Standard solution and quality control (QC) sample preparation

The stock solutions of morroniside, loganin and IS were prepared in methanol at a concentration of 1.57, 2.45 and 0.20 mg mL\(^{-1}\), respectively. Two series of working solutions were prepared by diluting respective stocking solution with methanol to suitable concentrations of 7.85–7850 ng mL\(^{-1}\) for morroniside and 6.12–6125 ng mL\(^{-1}\) for loganin. The IS stock solution was diluted with methanol to a final concentration of 200 ng mL\(^{-1}\).

To assay standard samples for construction of calibration curve, 30 μL mixture standard solution and 30 μL IS was added to 90 μL blank rat plasma. Then, the concentrations ranged from 1.96 to 1962.5 ng mL\(^{-1}\) for morroniside, 1.53–1531.25 ng mL\(^{-1}\) for loganin and 50 ng mL\(^{-1}\) for the IS, respectively. The quality control (QC) samples were prepared at low, medium, and high (9.81, 98.12, 981.25 ng mL\(^{-1}\) for morroniside and 7.66, 76.56, 765.62 ng mL\(^{-1}\) for loganin) concentrations in the same way as the standard calibration samples.
3. Results and discussion

3.1. Analysis method optimization

Different mobile phase compositions were investigated to acquire optimized response, suitable retention time and good peak shape. Methanol, acetonitrile and 0.1% formic acid in water were tested as possible mobile phases. As a result, acetonitrile and 0.1% formic acid in water was adopted as the solvent system with gradient elution. For the MS condition, both positive and negative scan modes were tested. Due to the more obvious fragment characteristic and higher sensitivity for morroniside and loganin, the negative mode was selected.

3.2. Validation procedures

3.2.1. Specificity and selectivity

The degree of interference by endogenous substances was assessed by inspection of chromatograms derived from processed blank and rat samples. The representative chromatograms from blank plasma, blank plasma spiked with morroniside, loganin and IS, and rat plasma obtained at 30 min after oral administration of shanzhuyu extract were displayed in Fig. 2. No endogenous interference or metabolites were observed at the retention times of morroniside (4.41 min) and loganin (4.86 min).

3.2.2. Linearity and lower limit of quantification (LLOQ)

The linearity of the calibration curve was determined by plotting the peak area ratio (y) of the analyte to the IS versus the nominal concentration (x) with weighted (1/x^2) least square linear regression. Representative calibration curves were as follows: 

\[ y = 0.0051x - 0.0022 \] (r=0.9988, morroniside) and 

\[ y = 0.0045x + 0.0048 \] (r=0.9976, loganin). The results showed that there was good correlation between the ratio of peak area and concentration for each compound within the test ranges. The LLOQ of the method was determined in six replicates by comparing the peak areas of the extracted samples with that of the unextracted standard solutions (n=6). The mean recoveries of morroniside and morro niside ranged from 67.62% to 78.23%. IS in rat plasma was 84.14 ± 2.96%. These results showed that the recoveries of these analytes were stable and reproducible.

For evaluation of matrix effect, peak areas of post-extraction blank plasma spiked with loganin and morroniside (B) were compared with those of loganin and morroniside neat solution in methanol at QC levels (A). The matrix effect was defined as the ratio of B/A × 100%. Matrix effect data (%) for morroniside in rat plasma was between 97.28 ± 6.56 and 102.75 ± 5.24. For loganin, the values ranged from 97.79 ± 7.98 to 102.21 ± 5.11. The matrix effect of IS was 95.07 ± 4.18. It can be concluded that no significant signal suppression or enhancement were observed in the present study. Recovery and matrix effect data of loganin and morroniside in rat plasma were shown in Table 3.

3.2.5. Stability

Based on peak areas in comparison with freshly prepared QC samples, the stability of QC samples at low, medium and high concentrations (n=6) exposed to different conditions (three successive freeze–thaw cycles; room temperature, 8 h; −80 °C, 14 days) were evaluated Table 4. The results indicated that loganin and morroniside were stable throughout all the stability tests.

3.3. Pharmacokinetic study

The method was successfully applied to investigate the pharmacokinetics of morroniside and loganin in plasma of normal and CKD rats after a single oral administration of shanzhuyu extract. The pharmacokinetic parameters were obtained by using the noncompartmental module of Drug and Statistic (DAS) 2.0 pharmacokinetic software. The mean plasma concentration-time profiles (n=6) were illustrated in Fig. 3 and the pharmacokinetic parameters analyzed were list in Table 5.

After oral administration of the shanzhuyu extract, the mean AUC_{0→t} of the CKD rats (564.20 ± 177.71 μg min mL\(^{-1}\) for morroniside and 765.91 ± 291.17 μg min mL\(^{-1}\) for loganin, p<0.05) were higher than that of normal rats (199.51 ± 21.96 μg min mL\(^{-1}\) for morroniside and 275.47 ± 82.06 μg min mL\(^{-1}\) for loganin, p<0.05). However, loganin and morroniside in the CKD group were eliminated more slowly than in normal group. The obvious lower CL/F (0.072 ± 0.012 vs 0.21 ± 0.063 L kg\(^{-1}\) min\(^{-1}\) for morroniside and 0.045 ± 0.013 vs 0.13 ± 0.049 L kg\(^{-1}\) min\(^{-1}\) for loganin, p<0.05) and higher T_{1/2} (196.65 ± 46.19 vs 92.57 ± 26.21 min for morroniside and 168.80 ± 70.13 vs 106.27 ± 44.41 min for loganin) compared with normal rat groups were observed.

The mean T_{max} was 30.50 ± 8.66 min in the model group,
morroniside and 1.45 ± 0.12 μg mL⁻¹ for loganin. These demonstrated much more morroniside and loganin after oral administration of Shanzhuyu extract were quickly absorbed in CKD model rats than in the normal rats, which might be beneficial for the therapy efficacy.

The CKD is known as renal impairment which may affect drug exposure in patients and appropriate dose adjustments might be required (Wang and Bajorek, 2014; Grewal et al., 2014; Moore et al., 2014; Stock, 2014; Vilchez et al., 2014). Renal impairment is associated with multiple physiological changes that affect the disposition of therapeutic drugs. The decreased clearance (CL) has been observed for drugs eliminated by renal and nonrenal pathways (Asconape, 2014; Lalande et al., 2014). The change may display the interplay between 2 major drug eliminating organs, the liver and the kidney. The down-regulation of liver CYP enzymes and transporters presumably by elevated levels of circulating inflammatory proteins, or direct inhibition of CYP enzymes and transporters by increased levels of circulating uremic toxins in patients with kidney disease have been postulated to describe the interrelationship between renal function and hepatic drug clearance (Dixon et al., 2014; Alishgorgan et al., 2015).

Oral bioavailability, i.e., the quantity of drug reaching the blood circulation, depends on different factors, the two most important being presystemic metabolism (intestinal and hepatic) and intestinal drug transport (Yeo et al., 2011; Li et al., 2012; Zhang et al., 2012; White et al., 2013; Hierro et al., 2014). Presystemic metabolism is primarily associated with intestinal and liver P450, whereas drug extrusion and import are mediated by several membrane proteins (transporters). The most important intestinal extrusion transporters are P-gp and MRP2, whereas the most important intestinal import transporter is the organic anion-transporting polypeptide (Oatp) type 3. Any modification in these systems will have important repercussions in the bioavailability of xenobiotics (Edwards and Ensom, 2012; Hierro et al., 2014). Based on above, the possible reasons for pharmacokinetic differences between normal and CKD rats may be addressed by the following explanations. Firstly, P-glycoprotein (P-gp, gene symbol ABCB1), one of the ATP-binding cassette transporter proteins, is considered as an important component of the blood–brain barrier, blood–placenta barrier, blood–testis barrier and other biological barriers in vivo (Naud et al., 2007; Kozlowska-Rup et al., 2014; Krishnamurthy et al., 2014). It has also been widely accepted that the intestinal P-gp can be an active secretion system or an absorption barrier by transporting some drugs from the intestinal cells into the lumen. The intestinal absorption of some iridoids glycoside, like geniposide, catalpol and loganin is enhanced with the inhibition of the intestinal P-gp activity (Li et al., 2008; Chula et al., 2012; Qosa et al. 2014). Thus, iridoids glycoside is probably a substrate of the intestinal P-gp and a decrease in intestinal P-gp could explain the increased bioavailability of drugs in chronic renal disease rats (Krishnamurthy et al., 2014). Furthermore, in this study, doxorubicin was used to establish CKD rats. However, doxorubicin showed hepatotoxicity by inducing toxicity and oxidative damage in liver tissues of rats (Feng et al., 2013; Singla et al., 2014; Wided et al., 2014). The liver plays a central role in the biotransformation of xenobiotics, including drugs. Some processes associated with hepatic disease states are, however, expected to affect drug disposition to a greater or lesser extent. It could contribute to decreased activity of drug metabolizing enzymes, changes in hepatic blood circulation and haemodynamics and so on (Dinan et al., 2014; Pfeifer et al., 2014; Yeung et al., 2014). Therefore, the harm of rat liver tissues might result in decreased metabolism of loganin and morroniside. Renal or hepatic disease can prolong the elimination of the parent drug or an active metabolite leading to accumulation and clinical toxicity. All of the above may synthetically result in the alteration of pharmacokinetic behavior of loganin and morroniside in CKD rats.

Table 3

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration (μg mL⁻¹)</th>
<th>Recovery (%)</th>
<th>Matrix effect (%)</th>
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</thead>
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<tr>
<td>Morroniside</td>
<td>9.81</td>
<td>69.10 ± 4.27</td>
<td>99.03 ± 3.34</td>
</tr>
<tr>
<td></td>
<td>98.12</td>
<td>67.62 ± 3.15</td>
<td>97.28 ± 6.56</td>
</tr>
<tr>
<td></td>
<td>981.25</td>
<td>76.54 ± 2.32</td>
<td>102.75 ± 5.24</td>
</tr>
<tr>
<td>Loganin</td>
<td>7.66</td>
<td>68.71 ± 5.46</td>
<td>102.21 ± 5.11</td>
</tr>
<tr>
<td></td>
<td>76.56</td>
<td>71.62 ± 2.97</td>
<td>99.37 ± 4.50</td>
</tr>
<tr>
<td></td>
<td>765.62</td>
<td>78.23 ± 3.03</td>
<td>97.79 ± 7.98</td>
</tr>
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Table 4

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration (μg mL⁻¹)</th>
<th>Three-freeze-thaw cycles (RSD%)</th>
<th>8 h at room temperature (RSD%)</th>
<th>14 days at −80°C (RSD%)</th>
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<tbody>
<tr>
<td>Morroniside</td>
<td>9.81</td>
<td>3.27</td>
<td>4.16</td>
<td>7.03</td>
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<tr>
<td></td>
<td>98.12</td>
<td>6.85</td>
<td>5.30</td>
<td>4.73</td>
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<td></td>
<td>981.25</td>
<td>5.73</td>
<td>6.19</td>
<td>7.31</td>
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<tr>
<td>Loganin</td>
<td>7.66</td>
<td>6.35</td>
<td>5.28</td>
<td>5.02</td>
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<tr>
<td></td>
<td>76.56</td>
<td>7.07</td>
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<tr>
<td></td>
<td>765.62</td>
<td>4.29</td>
<td>5.22</td>
<td>8.17</td>
</tr>
</tbody>
</table>

Fig. 3. Mean concentration-time curves in rat plasma after oral administration of shanzhuyu extract. (A) morroniside and (B) loganin.

60.50 ± 15.00 min in the normal group for morroniside. 45.00 ± 7.50 min in the model group, 60 ± 10.61 min in the normal group for loganin. The mean Cmax of the CKD rat was achieved with relatively high value of 2.81 ± 0.15 μg mL⁻¹ for morroniside and 3.11 ± 0.23 μg mL⁻¹ for loganin, which was detectable in normal rat plasma with low value of 1.32 ± 0.42 μg mL⁻¹ for morroniside and 1.45 ± 0.12 μg mL⁻¹ for loganin. These demonstrated much more morroniside and loganin after oral administration of Shanzhuyu extract were quickly absorbed in CKD model rats than in the normal rats, which might be beneficial for the therapy efficacy.
4. Conclusion

In this work, a sensitive, selective, and rapid UPLC–MS method has been developed and validated for the analysis of loganin and morroniside in normal and CKD rat plasma. The significant difference (p < 0.05) pharmacokinetic parameters includes AUC0–t, AUC0–∞, Cmax and CL2/F, which might partly result from the changes of P-gp and metabolic enzymes in the pathological state. The pharmacokinetic research in the pathological state might provide more useful information to guide the clinical usage of the medicine. Moreover, the method might be useful for other Chinese herb medicine.

Acknowledgements

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References


Table 5

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Morroniside (Normal rats)</th>
<th>Morroniside (CKD rats)</th>
<th>Loganin (Normal rats)</th>
<th>Loganin (CKD rats)</th>
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<tr>
<td>AUC0–t (μg min mL⁻¹)</td>
<td>199.51 ± 21.96</td>
<td>564.20 ± 177.71</td>
<td>275.47 ± 82.06</td>
<td>765.91 ± 291.17</td>
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<tr>
<td>AUC0–∞ (μg min mL⁻¹)</td>
<td>200.81 ± 23.87</td>
<td>592.90 ± 210.64</td>
<td>278.82 ± 77.51</td>
<td>811.40 ± 457.36</td>
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<tr>
<td>Cmax (μg mL⁻¹)</td>
<td>1.32 ± 0.42</td>
<td>2.81 ± 0.15</td>
<td>2.51 ± 0.13</td>
<td>3.11 ± 0.23</td>
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<tr>
<td>T1/2 (min)</td>
<td>60.50 ± 15.00</td>
<td>30.50 ± 8.66</td>
<td>41.44 ± 168.80</td>
<td>45.00 ± 7.50</td>
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<tr>
<td>T1/2 (min)</td>
<td>92.57 ± 26.21</td>
<td>196.65 ± 46.19</td>
<td>106.27 ± 41.44</td>
<td>168.80 ± 70.13</td>
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<td>CL2/F (l kg min⁻¹)</td>
<td>0.21 ± 0.063</td>
<td>0.072 ± 0.012</td>
<td>0.13 ± 0.049</td>
<td>0.045 ± 0.013</td>
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* p < 0.05 compared with normal rats.
Pak. J. Pharm. Sci. 27, 1891–1897.