doi.org/10.36721/PJPS.2023.36.6.REG.1729-1734.1

The effect of ritonavir on the pharmacokinetics of clonidine *in vivo* and *in vitro*

Peng Wang[1]* and Xiaoxia Hu[2]
[1]Department of Clinical Pharmacy, Jinhua People's Hospital (The Affiliated Jinhua Hospital of Wenzhou Medical University), Jinhua, Zhejiang, China.
[2]Department of Clinical Pharmacy, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, Zhejiang, China.

Abstract: This project aims to explore the repercussions of ritonavir on both the drug kinetics of clonidine in rats and clonidine metabolism in liver microsomes. Eighteen healthy male laboratory rats were haphazardly placed into groups: Group A, the control, Group B, got 20mg/kg ritonavir and Group C, got 180 mg/kg ritonavir. Ritonavir was administered to the rats by oral gavage and 30 minutes later, clonidine at 0.25mg/kg was administered for once. Moreover, rat and human liver microsomes, along with recombinant human CYP2D6*1, were used to study the inhibition effect of ritonavir on clonidine *in vitro*. The concentrations of clonidine and its metabolite were determined by the UPLC-MS/MS. The area under the curve (AUC) of clonidine increased ($P<0.01$) and clearance (CL) decreased significantly ($P<0.01$), after co-administration with 180mg/kg ritonavir. The half-maximal inhibitory concentration (IC_{50}) of ritonavir was 11.48μmol/L in rat liver microsomes, 3.52μmol/L in human liver microsomes and 18.04μmol/L in CYP2D6*1. Our findings demonstrate that ritonavir exhibited an inhibitory effect on clonidine metabolism *in vitro* and *in vivo*. It suggests that concurrent use of clonidine with ritonavir required close monitoring of the clonidine plasma concentration to alert drug adverse reactions.

Keywords: Clonidine, cytochrome P450, drug interaction, pharmacokinetics, ritonavir.

INTRODUCTION

Clonidine, the centrally acting sympatholytic agent, is generally approved for the treatment of hypertension (Mancia Chairperson *et al.*, 2023). The hypotensive effect of clonidine appears due to its action as an alpha2-adrenergic receptor agonist and its action on the imidazoline-I receptor (Bousquet *et al.*, 2020). Nevertheless, clonidine had a relatively narrow therapeutic window. Adverse effects to the therapeutic dose of clonidine are relatively common, particularly in specific patient groups using clonidine to treat medical conditions (Clavenna and Bonati, 2017; Viet-Quoc Nguyen *et al.*, 2022; Hanna *et al.*, 2022). Clonidine is mostly metabolized to inactive metabolites, 4-hydroxyclonidine, by the cytochrome P450 2D6 enzyme (CYP2D6) in the liver (Li *et al.*, 2019). Therefore, coad ministering clonidine with drugs that inhibit the activity of the CYP2D6 enzyme might lead to an increase in potential side effects.

Ritonavir, an antiretroviral agent, has been widely used for the treatment of viral infections such as COVID-19, SARS and HIV (McKee *et al.*, 2020; Jitta *et al.*, 2022). In addition, ritonavir is a potent and clinically important inhibitor of human CYP2D6 enzyme pathways (Agarwal and Agarwal, 2021). Previous research has shown that ritonavir or ritonavir-containing antiviral drugs can significantly increase blood levels of psychotropic medications that are primarily eliminated by CYP2D6 metabolism in humans and increase the chance of psychotropic adverse drug reactions (e.g. fluoxetine, duloxetine) (Mansuri *et al.*, 2020).

Viral infections such as COVID-19, SARS and HIV are becoming severe global public health problems in the current century. Hypertension was among the most prevalent underlying diseases among patients with viral infections and was associated with increased mortality and disease progression in virus-infected patients (Masenga *et al.*, 2020; Emami *et al.*, 2020; Pranata *et al.*, 2020). Therefore, concomitant administration of both antihypertensive drugs and antiviral treatment drugs, such as ritonavir and clonidine, can be expected. We should pay close attention to the drug-drug interaction between ritonavir and clonidine. The main objective of the current investigation is to assess the inhibitory capacity of ritonavir on clonidine metabolism. We evaluated ritonavir's impact on clonidine in rat and human hepatic microsome models. Furthermore, the influence of ritonavir on clonidine pharmacokinetics in rats was also investigated. We hope that our study could provide a basis for the safety of the clinical medication.

MATERIALS AND METHODS

Chemicals and reagents

Ritonavir was obtained from Canspec (Shanghai, China). Clonidine and 4-hydroxyclonidine were sourced from TRC (Toronto, Canada). NADPH and Losartan were supplied by Sigma (St. Louis, MO, USA). Acetonitrile and formic acid, both meeting HPLC specifications, were

*Corresponding author: e-mail: 754952527@qq.com

produced by Merck KGaA (Darmstadt, Germany). Human liver microsomes was purchased from Corning Life Sciences (NY, USA). Recombinant human CYP2D6*1 and cytochrome b5 were kind gifts from Beijing Hospital (Beijing, China). The ultrapure water utilized in the experiment was provided via the Millipore Milli-Q system (Bedford, USA). Dimethyl sulfoxide was produced by Wenzhou Changfeng Biotechnology Co. Ltd. (Wenzhou, China). Analytical grade reagents were used throughout this study.

Equipment and conditions

Clonidine in plasma concentrations and 4-hydroxyclonidine formed during microsomal incubations were analyzed using a validated UPLC-MS/MS. The analysis system consisted of ACQUITY UPLC H-Class and XEVO TQD triple-quadrupole mass spectrometer, both manufactured by Waters Corporation (Massachusetts, USA). Chromatographic separation was achieved with a 1.7μm Acquity BEH C18 column (2.1 mm×50 mm, Waters, USA) and gradient elution using a mobile phase consisting of ultrapure water A (containing 0.1% formic acid) and organic phase B (acetonitrile). The ratio changed as follows: 0-0.5 min (70-10% A), 0.5-1.5 min (10-10% A), 1.5-1.8 min (10-70% A), 1.8-3 min (70 % A). Experiment flow rate: 0.2 mL/min; Total elution time: 3.0 min. The column temperature was maintained at 40ºC. A 2μL of processed biological sample was injected into UPLC-MS/MS for sample analysis. Following electrospray ionization, mass spectrometry was conducted using MRM at unit resolution in positive mode, with a 63 ms settling period. Nitrogen was employed for desolvation gas at a flow rate of 1000 L/h and as cone gas at 50L/h. Experimental parameters included a capillary voltage of 2.99kV, a source temperature set at 150ºC and a desolvation temperature of 498ºC. Clonidine (m/z 230.06/160.02, retention time 0.66min), 4-hydroxyclonidine (m/z 246.15/176.05, retention time 0.65 min) and Losartan (m/z 423.2/207.2, retention time 2.36 min). The calibration curves were linear across the range of 5-1,000pg/mL in rat plasma for clonidine and the range of 0.1-200μg/mL in microsomes for 4-hydroxyclonidine, both with a regression coefficient (R_2)>0.99.

In vivo pharmacokinetic studies

Experimental animals

Eighteen healthy male Sprague-Dawley rats, averaging 5 months in age and bodyweight 230-270g, were sourced from the Animal Research Institute at Wenzhou Medical University. The purchased experimental rats were acclimated in a controlled environment for a minimum of 7 days before the experiment. This environment was kept at a consistent temperature of 20-26°C, with humidity at 55-65% and adhering to a 12-hour light/dark cycle. During this period, the rats had unrestricted access to tap water and standard rat food. Additionally, they were subjected to a 12-hour fasting period with access to water ad libitum immediately before the experiment. Wenzhou Medical University Ethics Committee approved the animal experiments (wydw2018-0002). Furthermore, the experiment was conducted following the protocols for the ethical treatment of animals.

Pharmacokinetic experiment

Experimental animals were randomly allocated into three groups. Group A received a comparable volume of a 0.5% CMC-Na solution to serve as a control for comparison. Group B was administered ritonavir orally with 20mg/kg, while Group C was treated orally with ritonavir 180mg/kg. Ritonavir in both treated groups was prepared using 0.5% CMC-Na solution. Following the administration of ritonavir, clonidine (0.25mg/kg dissolved by 0.9 % NaCl) was administered by the oral route using gavage to all rats in each group. The dose of medicine administered to rats in this study was calculated according to a clinically relevant maximum human dose adjusted for body surface area. Blood samples (300μL) were drawn into a heparin-pretreated tube via tail vein as follows: 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 12h after the clonidine administration. Samples were chilled and centrifuged at 16,200g for 10 min and all plasmas were isolated and preserved at -80ºC until assay.

Biological specimen processing

30μL of internal standard (losartan, 0.4μg/mL dissolved by methanol) was combined with 100μL rat plasma and vortex mixed for 10 sec. A protein precipitation method was employed to extract the clonidine and losartan by adding 200μL of acetonitrile. After vortexing the pooled plasma samples for 2 min and centrifuging at 16,200g for 10 min, the resulting supernatant (100μL) was mixed with ultra-pure water in the ratio of 1: 1 v/v. Subsequently, A 2μL resulting solution was introduced into the UPLC-MS/MS for qualitative and quantitative analysis.

In vitro metabolic investigation

Rat hepatic microsome isolation

The fresh rat livers were collected for gravimetric determination, homogenized and mixed in refrigerated 0.01 mmol/L PBS with a sucrose concentration 0.25 mmol/L and then transferred to polypropylene centrifuge tubes. After centrifuging for 30 min at 10,000g, the resulting supernatants were collected in new tubes and subjected to another 30min centrifugation at 10,000g. Subsequently, the supernatants underwent a final centrifugation at 100,000g for 60min. The resulting microsomal precipitation was washed and resuspended in chilled 0.01 mmol/L PBS and microsomal protein levels were assessed using Bradford Protein Assay Kit (Wang *et al.*, 2015).

In vitro assessment of ritonavir on clonidine metabolism

The incubation conditions for this study, including reaction time and microsomal concentration, have been optimized before the experiment. Each incubation tube

contained 0.66mg/mL rat liver microsomes (or 0.44mg/mL human liver microsomes, or 5 pmol CYP2D6*1 + 5 pmol cytochrome b5), along with 1 mmol/L NADPH, 50μmol/L clonidine, varying concentrations of ritonavir (ranging from 0.01 to 100μmol/L). Lastly, the incubation system was completed by adding a 100 mmol/L Tris-HCl (pH 7.4) solution, bringing the final volume to 200μL. The drug concentration and microsomal protein concentration in the system were the final concentrations. After a brief 5 min equilibration at 37ºC, the incubation process was triggered by introducing an NADPH solution. The incubation was conducted at 37ºC for 30 min and stopped by immediately putting the incubation tube in an ice-water bath, followed by adding 30μL internal standard (1μg/mL) and 400μL acetonitrile. The mixed solution was vortexing and centrifugation. The supernatant was diluted with ultrapure water and introduced into the UPLC-MS/MS to measure the 4-hydroxyclonidine concentration. Microsomal incubation aimed to assess ritonavir's inhibitory impact on clonidine metabolism. The evaluation of the inhibition was based on the conversion rate of clonidine to 4-hydroxyclonidine with 100μmol/L of ritonavir in rat liver microsomes and human liver microsomes. The IC_{50} value was calculated using a dose-response curve generated from the data obtained by varying ritonavir concentrations.

STATISTICAL ANALYSES

GraphPad Prism version 6.0 (Graphpad Software Inc., USA) was utilized for constructing the dose-response curve and for statistical analysis to determine the IC_{50} value. DAS version 3.0 (Bontz Inc., China) was employed to assess the pharmacokinetic parameters of clonidine through standard non-compartmental methods. Pharmacokinetic parameters and inhibition rates among the experimental groups were evaluated by one-way ANOVA, performed with SPSS 19.0 (SPSS Inc., USA). Data: mean ± SD; Significance: $P<0.05$, high significance: $P<0.01$.

RESULTS

Effect of ritonavir on the pharmacokinetics of clonidine in vivo

Mean plasma concentration-time curves of clonidine following oral dosing of 0.25mg/kg clonidine in the control and co-administration with ritonavir (20 and 180mg/kg) in rats are depicted in fig. 1. The pharmacokinetic values obtained after the oral administration of clonidine alone and in the presence of 20 and 180mg/kg of ritonavir are summarized in table 1. There were some changes in several pharmacokinetic parameters in the ritonavir-treated rats. For instance, rats treated with high dose ritonavir (Group C) exhibited an increase in AUC_{0-t} ($P < 0.01$), $AUC_{0-\infty}$ ($P < 0.01$) and C_{max} for plasma clonidine by 68, 98 and 36%, respectively; and it showed a decrease in clonidine CLz/F ($P<0.01$) and Vz/F by 48 and 57%. In low-dose ritonavir-treated rats (Group B), there was a slight increase in clonidine AUC and C_{max} and a slight decrease in clonidine CLz/F and Vz/F, however, these were not statistically significant. The other pharmacokinetic parameters, namely MRT, $t_{1/2}$ and T_{max} did not exhibit significant differences among the treatment groups.

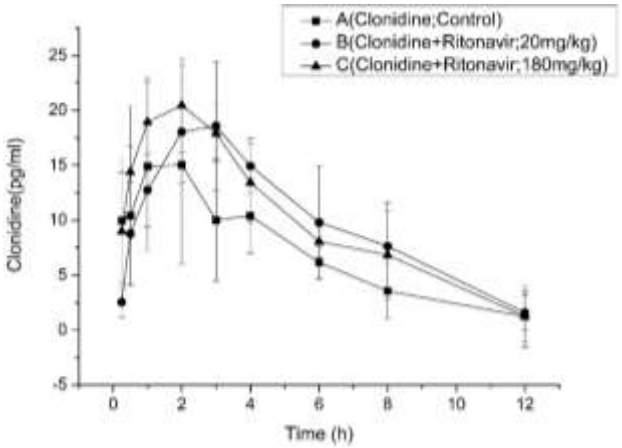

Fig. 1: Mean plasma concentration-time profiles clonidine in the presence and absence of ritonavir in Sprague–Dawley male rats. (■) control group, (●) a single dose of 20mg/kg ritonavir, and (▲) a single dose of 180 mg/kg ritonavir. Bars represent the standard deviation (n = 6). The data are expressed as mean±SD.

In vitro ritonavir impact on clonidine metabolism

After co-administration 100μmol/L ritonavir, the rate of 4-hydroxyclonidine generation decreased to 26.26% in rat liver microsomes, 25.49% in human liver microsomes and 32.81% in CYP2D6*1, as shown in fig. 2. Furthermore, the IC_{50} of ritonavir in rat, human liver microsomes and CYP2D6*1, were estimated to be 11.48μmol/L, 3.52μmol/L and 18.04μmol/L respectively (fig. 3).

DISCUSSION

CYP450 enzyme isoforms are jointly responsible for clonidine metabolism, the predominant metabolic pathway is the 4-hydroxylation of clonidine, primarily catalyzed by CYP2D6 (Li *et al.*, 2019). Interindividual genetic differences and potential drug-drug interactions that affect CYP2D6 activities should regarded as crucial factors influencing the efficacy and safety of clonidine. In our previous study, we conducted a comprehensive evaluation of how 22 newly discovered CYP2D6 variants impact the metabolism of clonidine (Li *et al.*, 2019). Therefore, the present study evaluated how ritonavir, a CYP2D6 inhibitor, affects the pharmacokinetics of clonidine in rats to investigate the potential drug interaction between clonidine and ritonavir via the inhibition of CYP2D6.

Table 1: Pharmacokinetic parameters for clonidine in rats pretreated with or without ritonavir followed by single dose clonidine (n=6)

Parameters	unit	A	B	C
$AUC_{(0-t)}$	ng/L*h	83.69±22.94	118.19±25.14	140.75±18.72 **
$AUC_{(0-\infty)}$	ng/L*h	89.45±18.71	124.88±29.77	177.14±59.21 **
$MRT_{(0-t)}$	h	4.09±1.52	4.39±0.79	4.77±0.64
$MRT_{(0-\infty)}$	H	5.05±2.47	4.92±1.28	6.72±4.10
$t_{1/2z}$	H	2.22±2.06	2.08±1.06	2.34±2.05
T_{max}	H	2.00±1.23	3.00±0.71	2.80±1.30
Vz/F	L/kg	9,930.82±9,888.21	5,951.43±2,525.05	4,232.30±2,664.74
CLz/F	L/h/kg	2,900.87±642.18	2,115.72±611.46	1,512.47±384.48 **
C_{max}	ng/L	18.08±6.79	21.27±5.17	24.56±9.62

Note: The data are expressed as mean±SD; group A was the control group, group B received a dose of 20mg/kg ritonavir, and group C received a dose of 180mg/kg ritonavir. AUC area under the concentration-time curve; MRT mean residence time; $t_{1/2z}$ elimination half-life; T_{max} time required to reach C_{max}; Vz/F volume of distribution in terminal phase; CLz/F apparent clearance; C_{max} maximum plasma concentration. * Significant difference, P<0.05; **Highly significant difference, P<0.01.

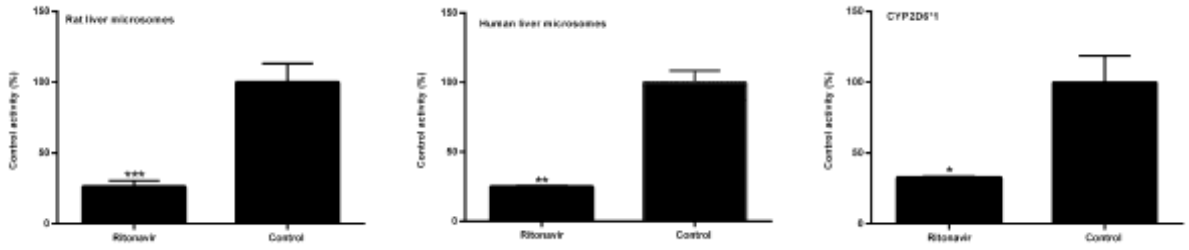

Fig. 2: Inhibition effect of ritonavir (100μmol/L) on 4-hydroxyclonidine formation in rat liver microsomes (A), human liver microsomes (B), and CYP2D6*1 (C). Control activity was measured by the generating rate of 4-hydroxyclonidine in the presence of 100μmol/L ritonavir compared with control (no inhibitor). The data are expressed as mean ± SD, n=3. *Significant difference, P<0.05; **Significant difference, P<0.01; ***Significant difference, P<0.001.

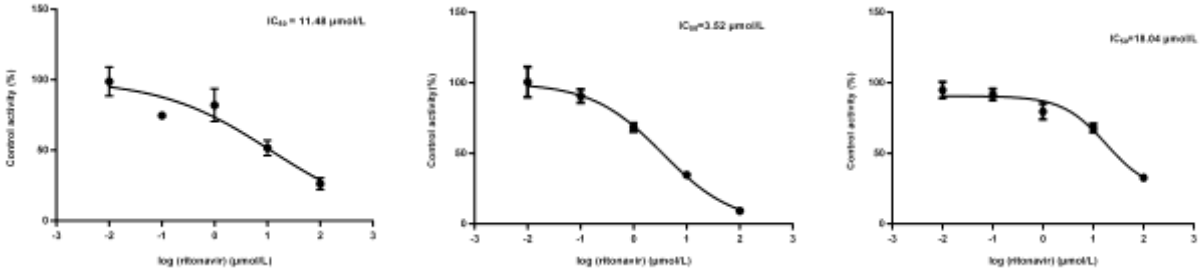

Fig. 3: Inhibition of 4-hydroxyclonidine formation by ritonavir in rat liver microsomes (A), human liver microsomes (B) and CYP2D6*1 (C). Control activity was measured by the generating rate of 4-hydroxyclonidine in the presence

The presence of ritonavir significantly increased the AUC of co-administered clonidine, possibly due to the significantly slower CL/F of clonidine with ritonavir. Moreover, the AUC of clonidine increased by 40-98 % in the rats co-administered with 20-180mg/ kg of ritonavir, then the C_{max} of clonidine increased by 18-36%. The AUC and C_{max} of clonidine increased as increasing the dose of ritonavir from 20-180mg/kg. These results are similar to the observation from the previous studies with venetoclax and elvitegravir. They are metabolized primarily by the CYP450 enzyme, like clonidine. The previous study has demonstrated that a single 50 or 100mg ritonavir leads to 2.3- to 2.4-fold increase in the venetoclax C_{max} and 6.1- and 8.1-fold in AUC, with the magnitude of the increase varying depending on the ritonavir dose (Freise *et al.*, 2018). Then the plasma AUC and C_{max} of elvitegravir increased with increasing doses of ritonavir (doses of 20, 50, 100 and 200mg) (Mathias *et al.*, 2009). Thus, we speculated that ritonavir could impede the metabolism of clonidine in rats, with the inhibitory impact showing dose

dependency within a defined range. However, the correlation requires further experimental verification.

In vitro results additionally demonstrated ritonavir's inhibitory impact on the liver metabolism of clonidine, which aligns well with the pharmacokinetics result *in vivo*. In CYP2D6*1, ritonavir exhibited a 32.81% metabolic inhibition rate for clonidine, with an IC_{50} value of 18.04μmol/L, suggesting that ritonavir inhibits clonidine metabolism possibly by inhibiting CYP2D6. Furthermore, ritonavir has almost equivalent inhibitory effects on clonidine metabolism both in rat and human liver microsomes (26.26 % VS 25.49 %). The IC_{50} of ritonavir for inhibition action in rat liver microsomes was close to that in human liver microsomes. Hence, we hypothesized that ritonavir may exhibit similar metabolic inhibition effect on clonidine in both rats and humans and this inhibitory effect is likely achieved through the inhibition of CYP2D6.

The most common adverse reactions to clonidine were central nervous system depression, hypotension and dry mouth (Derk *et al*., 2022). Pharmacodynamic studies of clonidine have demonstrated the drug effect of clonidine, such as degree of sedation, fall in blood pressure and reduction in salivary flow is closely related to plasma clonidine concentrations (Isbister *et al.*, 2017; Dollery *et al.*, 1976). In practical terms, this means that the increased plasma drug exposure of clonidine attributable to ritonavir coadministration may be accompanied by the enhanced pharmacodynamic activity of clonidine. Therefore, exposure to ritonavir during treatment in patients receiving clonidine will increase the potential for adverse reaction and caution regarding the possible hazards of this combination appears to be warranted.

CONCLUSION

In summary, the studies reported herein are the first to report that coadministration of ritonavir could inhibit CYP2D6-mediated clonidine metabolism and change pharmacokinetic behavior. These results of the pharmacokinetic study are also supported by the CYP inhibition and metabolism *in vitro* study. However, further studies are required to elucidate the restraining influence of ritonavir on clonidine metabolism in humans.

REFERENCES

Agarwal S and Agarwal SK (2021). Lopinavir-ritonavir in SARS-CoV-2 infection and drug-drug interactions with cardioactive medications. *Cardiovasc. Drugs Ther.*, **35**(3): 427-440.

Bousquet P, Hudson A, García-Sevilla JA and Li JX (2020). Imidazoline receptor system: The past, the present and the future. *Pharmacol. Rev.*, **72**(1): 50-79.

Clavenna A and Bonati M (2017). Pediatric pharmacoepidemiology - safety and effectiveness of medicines for ADHD. *Expert Opin. Drug Saf.*, **16**(12): 1335-1345.

Derk G, Barton A, An R, Fang HY, Ashrafi SA and Wilund K (2022). The safety and efficacy of clonidine in hemodialysis patients: A systematic review and meta-analysis. *Pharmacology*, **107**(11-12): 545-555.

Dollery CT, Davies DS, Draffan GH, Dargie HJ, Dean CR, Reid JL, Clare RA and Murray S (1976). Clinical pharmacology and pharmacokinetics of clonidine. *Clin. Pharmacol. Ther.*, **19**(1): 11-17.

Emami A, Javanmardi F, Pirbonyeh N and Akbari A (2020). Prevalence of underlying diseases in hospitalized patients with Covid-19: A systematic review and meta-analysis. *Arch. Acad. Emerg. Med.*, **8**(1): e35.

Freise KJ, Hu B and Salem AH (2018). Impact of ritonavir dose and schedule on CYP3A inhibition and venetoclax clinical pharmacokinetics. *Eur. J. Clin. Pharmacol.*, **74**(4): 413-421.

Hanna J, Ghazi L, Yamamoto Y, Simonov M, Shah T, Wilson FP and Peixoto AJ (2022). Excessive blood pressure response to clonidine in hospitalized patients with asymptomatic severe hypertension. *Am. J. Hypertens.*, **35**(5): 433-440.

Isbister GK, Heppell SP, Page CB and Ryan NM (2017). Adult clonidine overdose: prolonged bradycardia and central nervous system depression, but not severe toxicity. *Clin. Toxicol. (Phila.)*, **55**(3): 187-192.

Jitta SR, Salwa, Bhaskaran NA, Marques SM and Kumar L (2022). Recent advances in nanoformulation development of ritonavir, a key protease inhibitor used in the treatment of HIV-AIDS. *Expert Opin. Drug Deliv.*, **19**(9): 1133-1148.

Li X, Hu X, Yang F, Yuan L, Cai J and Hu G (2019). Effects of 24 CYP2D6 variants found in Chinese population on the metabolism of clonidine *in vitro*. *Chem. Biol. Interact.*, **313**: 108840.

Mancia Chairperson G, Kreutz Co-Chair R, Brunström M, Burnier M, Grassi G, Januszewicz A, Muiesan ML, Tsioufis K, Agabiti-Rosei E, Algharably EAE, Azizi M, Benetos A, Borghi C, Hitij JB, Cifkova R, Coca A, Cornelissen V, Cruickshank K, Cunha PG, Danser AHJ, de Pinho RM, Delles C, Dominiczak AF, Dorobantu M, Doumas M, Fernández-Alfonso MS, Halimi JM, Járai Z, Jelaković B, Jordan J, Kuznetsova T, Laurent S, Lovic D, Lurbe E, Mahfoud F, Manolis A, Miglinas M, Narkiewicz K, Niiranen T, Palatini P, Parati G, Pathak A, Persu A, Polonia J, Redon J, Sarafidis P, Schmieder R, Spronck B, Stabouli S, Stergiou G, Taddei S, Thomopoulos C, Tomaszewski M, Van de Borne P, Wanner C, Weber T, Williams B, Zhang ZY and Kjeldsen SE (2023). 2023 ESH Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension

Endorsed by the European Renal Association (ERA) and the International Society of Hypertension (ISH). *J Hypertens*.

Mansuri Z, Shah B, Adnan M, Chaudhari G and Jolly T (2020). Ritonavir/lopinavir and its potential interactions with psychiatric medications: A Covid-19 perspective. *Prim. Care Companion CNS Disord.*, **22**(3): 20com02677.

Masenga SK, Elijovich F, Koethe JR, Hamooya BM, Heimburger DC, Munsaka SM, Laffer CL and Kirabo A (2020). Hypertension and metabolic syndrome in persons with HIV. *Curr. Hypertens. Rep.*, **22**(10): 78.

Mathias AA, West S, Hui J and Kearney BP (2009). Dose-response of ritonavir on hepatic CYP3A activity and elvitegravir oral exposure. *Clin. Pharmacol. Ther.*, **85**(1): 64-70.

McKee DL, Sternberg A, Stange U, Laufer S and Naujokat C (2020). Candidate drugs against SARS-CoV-2 and COVID-19. *Pharmacol. Res.*, **157**: 104859.

Pranata R, Lim MA, Huang I, Raharjo SB and Lukito AA (2020). Hypertension is associated with increased mortality and severity of disease in COVID-19 pneumonia: A systematic review, meta-analysis and meta-regression. *J. Renin Angiotensin Aldosterone Syst.*, **21**(2): 1470320320926899.

Viet-Quoc Nguyen P, Le Berre C, Fillion L and Lafleur M (2022). Safety and efficacy of clonidine for acute hypertensive urgency in an older and hospitalized population. *Sr. Care Pharm.*, **37**(4): 157-162.

Wang Z, Sun W, Huang CK, Wang L, Xia MM, Cui X, Hu GX and Wang ZS (2015). Inhibitory effects of curcumin on activity of cytochrome P450 2C9 enzyme in human and 2C11 in rat liver microsomes. *Drug Dev. Ind. Pharm.*, **41**(4): 613-616.