RESEARCH ARTICLE

High performance liquid chromatography-mass spectrometry/mass spectrometry (HPLC-MS/MS) analysis of transmembrane transport characteristics of nuciferine in Caco-2 cells

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Nuciferine is an aporphine alkaloid extracted from lotus leaves, which has the functions of reducing fat, losing weight, and resisting oxidation, *etc*. However, the bioavailability of nuciferine is very low, which seriously affects the pharmacological effects of nuciferine. In order to provide theoretical basis for the clinical use of nuciferine, this study focused on the transmembrane transport characteristics of nuciferine and its influencing factors. Based on establishing a HPLC-MS/MS quantitative method for nuciferine in the cell culture, the Caco-2 monolayer cell culture was established by trans-well, and the transmembrane transport characteristics and influencing factors of nuciferine were clarified. The Caco-2 cell line was cultured in trans-well, and 500, 2,500, and 10,000 ng/mL concentration groups were set up. Samples were taken from the basolateral side (BL side) at 0.5 and 1.0 h, respectively, for liquid chromatograph tandem mass spectrometer (LC-MS/MS) detection, and the apparent permeability coefficients (Papp) from apical side to basolateral side (AP-BL) and from basolateral side to apical side (BL-AP) were calculated, respectively. Besides, different pH treatment groups, verapamil, tetraethylamine, chlorogenic acid, caffeic acid, and ouabain treatment groups were all set to calculate the apparent permeability coefficient of nuciferine. Papp of high concentration treatment group was significantly lower than that of medium and low concentration treatment groups, which indicated that the transmembrane transport of nuciferine was related to concentration. The transport of nuciferine across membrane was significantly affected by pH value of culture medium with lowering the pH value could significantly promote the transportation of nuciferine. Chlorogenic acid and caffeic acid significantly promoted the transport of nuciferine, while ouabain had no significant effect on it. Tetraethylamine significantly inhibited the transport of nuciferine across membranes. The transmembrane transport characteristics of nuciferine are mainly passive transport. Drug transporters have significant influence on nuciferine transmembrane transport, which may be related to organic cation transporters (OCT), while P-glycoprotein has no significant influence on nuciferine efflux transport. The results of this study provide a new idea for improving the bioavailability of nuciferine and further exerting the pharmacological effects of nuciferine.

Keywords: nuciferine; LC-MS/MS; Caco-2 cell; trans-membrane transport.

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Introduction

Lotus leaf is the dry leaf of *Nymphaeaceae* plant *Nelumbo nucifera Gaertn*. Traditional medicine shows that lotus leaf has bitter taste and flat nature and has the functions of removing heat and promoting diuresis [1-3]. Nuciferine is an active ingredient extracted from lotus leaf [4].

Studies have shown that nuciferine has many pharmacological effects, such as lipid-lowering [5, 6], anti-inflammatory [7], and anti-tumor [8-10]. In recent years, the study on the pharmacokinetics of nuciferine showed that nuciferine was absorbed rapidly *in vivo* [11, 12] and easily metabolized by drug metabolism enzymes in liver microsomes [13-15]. However, there are few reports on the intestinal absorption mechanisms and characteristics of nuciferine. Shi *et al*. studied the effect of nuciferine on intestinal permeability in mice [16], and Zhang *et al.* studied a potential molecular mechanism of the anti-inflammatory effects of nuciferine in lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 cells [17]. However, they only preliminarily studied the absorption difference of nuciferine at the intestinal and cellular levels rather than carrying out relevant research from the angle of absorption influencing factors and drug combination application. Caco-2 cell is a kind of human cloned colon adenocarcinoma cell, which is similar to small intestinal epithelial cells in structure and function and is often used to study the mechanism of drug transport. In this study, Caco-2 cells were used as the experimental model to study the transmembrane transport characteristics of nuciferine and its influencing factors, which provided theoretical basis for further elucidating the transmembrane transport mechanisms of nuciferine and the combination of compound drugs.

Material and methods

HPLC-MS/MS method establishment and validation

The Agilent 6460 Triple Quad high performance liquid chromatography-mass spectrometry system (Agilent, Santa Clara, CA, USA) featured by Waters T3 column (1.6 µm, 100 mm × 2.1 mm) was employed for this study. The mobile phase involved 0.1% formic acid-water (A) and methanol (B) elution gradient solutions was set as at the points of 0 min 15% B, 1 min 80% B, 3 min 15% B, 5min stop with the injection volume of 1.0 µL. Mass spectrometry parameters were set to positive ion mode, MRM scanning and monitoring. The gas curtain gas flow rate was 35 L/min, collision gas was 8 L/min, electrospray voltage was 5.5 KV, ion source temperature was 550℃, atomizer and auxiliary gas were both 160 psi, de-clustering voltage was 69 V, inlet voltage was 6 V, collision voltage was 13 V and outlet voltage was 10 V. Ion pairs for detection was set as nuciferine 296.3→265.2 and the internal standard phenacetin 180.2→110.1.

Nuciferine (purity over 98%) was purchased from Sichuan Weikeqi Biotechnology Co., Ltd. (Chengdu, Sichuan, China), and phenacetin (as internal standard, purity ≥99%) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). 0.1 mL nuciferine solution of 10, 50, 100, 500, 1,000, and 5,000 ng/mL was accurately prepared with blank Caco-2 cell culture medium, and then, 10 µL phenacetin solution with a concentration of 200 ng/mL was added followed by adding 3 mL ethyl acetate for shaking extraction. After centrifugation at 10,000 g for 5 mins at 4℃, the organic phase was collected and dried with nitrogen blowing instrument at 45°C water bath. The residue was reconstituted in 0.1 mL of chromatographic methanol, ultrasonic oscillated, centrifuged again at 10 000 g for 5 mins at 4℃, and then, the supernatant was taken for analysis. Finally, the standard curve was drawn with the mass concentration of nuciferine. The peak area ratio of nuciferine to internal standard, the standard curve equation, and correlation coefficient (r) were obtained.

The quality control samples were prepared from blank culture media with the nuciferine concentrations of 10, 100, and 1,000 ng/mL, and were placed at 4℃ and -20℃ for 12h and 24h, respectively. The samples were analyzed, and the ratio of nuciferine and its internal standard peak area was substituted into the standard curve equation. The stability of nuciferine in the samples was calculated by comparing the ratio of calculated nuciferine concentration with the added nuciferine concentration.

The nuciferine methanol solutions with corresponding low, medium, and high concentrations as above were prepared at the same time. Samples were injected for analysis. The peak area of nuciferine measured in quality control samples was compared to that in methanol solution with the same concentration, and the recovery rate was calculated. In addition, 10, 100, and 1,000 ng/mL nuciferine concentrations quality control samples were injected separately. The ratio of the measured nuciferine to the peak area of the internal standard was substituted into the standard curve. The calculated nuciferine concentration was compared to the concentration of nuciferine added in the quality control samples, and the accuracy of the method was obtained. Variation coefficients of nuciferine areas in the samples were measured through 5 independent detections in an intra-day and inter-day manner for evaluating precision of above established HPLC-MS/MS method.

Detection of cell density

Caco-2 cells were purchased from National collection of authenticated cell cultures (Shanghai Cell Bank, Chinese Academy of Sciences, Shanghai, China). The reagents including fetal bovine serum (FBS), DMEM medium, nonessential amino acids, streptomycin, trypsin, and phosphate buffer saline (PBS) were purchased from Gibco products (Thermo Fisher Scientific, Waltham, MA, USA). Trans-well chamber (Corning products) was purchased from Hangzhou Lanbao Haibo Biotechnology co., Ltd. (Hangzhou, Zhejiang, China). Trans-well cell model with transmembrane resistance above 200 Ω/cm^2, phenol red permeability below 10%, and alkaline phosphatase activity on apical (AP) side 10 times higher than that on basolateral (BL) side was adopted for this experiment. Before the experiment, the cells were washed three times with preheated PBS buffer solution (37℃), and the last time was incubated in an incubator at 37℃ for 30 mins. The buffer solution was gently sucked off to wash away impurities on the surface of Caco-2 cells.

Two trans-well sterile chambers were placed in the middle of the plate and marked. The cells were removed from the incubator, and the original culture solution was sucked out. The metabolite was washed with PBS buffer solution, and then, 1-2 mL of digestive solution (EDTA and phenol red) was added for enzymatic hydrolysis. After 2-3 minutes, when the cells became round and there were signs of shedding, the digestive solution was discarded, and the culture solution was added. The culture solution was blown with glass pipet to make the cells enter the culture solution. After mixing well, 400 µL of culture solution containing cells was added to the upper chamber (AP side) of each model, and then, the inoculated Trans-Well chamber was put into a CO_2 incubator for culture. After 21 days, the original culture medium was discarded, 200 µL phenol red solution (3.5 µg/mL) was added to AP side, while 400 µL of blank PBS buffer was added to BL side. 100 µL of PBS buffer was absorbed from BL side at 0.5, 1.0, 1.5, and 2.0 h, respectively. Then 100 µL of blank PBS buffer was added. After sampling, the concentrations of phenol red in the upper (AP) and lower (BL) chambers were determined by HPLC, and then substituted into the standard curve to obtain the concentrations of phenol red in the upper chamber and lower chamber for phenol red transmittance calculation.

Transmembrane transport of nuciferine in Caco-2 cells

Caco-2 cells that reached the compactness requirement in Trans-well chamber were selected (the transmittance of phenol red in Trans-well chamber was less than 3% within 0-2 hours). The culture solution in the chamber was sucked, and the cells were washed with PBS buffer for 3 times. For AP to BL chambers, 200 µL of low, medium, and high concentration (500, 2500, 10,000 ng/mL) nuciferine solution and 2,500 ng/mL of nuciferine combined with verapamil (150 ng/mL) mixture were added to AP side as supply pool, and 400 µL of PBS buffer was added to BL side as receiving pool. For BL to AP chambers, 200 µL of low, medium, and high concentration nuciferine solution and mixture of

nuciferine and verapamil were added to BL side as supply pool, and 400 µL of PBS buffer was added to AP side as receiving pool. 100 µL of liquid was taken from the receiving pool at 0.5 and 1.0 h, respectively, and 100 µL of PBS buffer was added to the receiving pool. The transport quantity of nuciferine in the sample was detected by LC-MS/MS. The apparent permeability coefficient (Papp) was calculated.

Influencing factors of nuciferine transmembrane transport on Caco-2 cells

Verapamil, chlorogenic acid, caffeic acid, and tetraethylamine were all analytically pure and provided by Hangzhou Lanbao Haibo Biotechnology Co., Ltd. (Hangzhou, Zhejiang, China). Phosphate buffers with pH values of 6.0, 6.5, and 7.0 were used to prepare nuciferine solution, while PBS buffer was used to prepare chlorogenic acid (1,000 ng/mL) nuciferine (2,500 ng/mL) mixed solution, caffeic acid (1,000 ng/mL) nuciferine (2,500 ng/mL) mixed solution, ouabain (10 µg/mL) nuciferine (2,500 ng/mL) mixed solution, and tetraethylamine (25 µg/mL) nuciferine (2,500 ng/mL) mixed solution.

Caco-2 cells were inoculated in a culture plate with a cell concentration of 1×10^5/mL. The cells were cultured in a constant temperature incubator at 37°C. After two weeks of continuous culture, cells were used for the experiment of nuciferine transport. The culture medium was changed every two days in the first week and every day in the second week. Before the experiment, the protocell culture solution was discarded. The cells were washed with PBS buffer for 3 times. The above-mentioned nuciferine solution was added and mixed with cells, and then, the plate was put back to CO_2 incubator for culture. The culture plate was taken out at 0.5 h and 1.0 h. 100 µL of liquid from the receiving pool was sucked as a sample, and 100 µL of PBS buffer was added in the receiving pool. The sample was treated according to the HPLC-MS/MS detection method mentioned above. Then, the mixed solution containing nuciferine was sucked out. Pre-cooled PBS solution was quickly added to the culture plate to stop the cell transferring nuciferine. The cells were then washed with PBS for 3 times. 2 mL of ultrapure water was added to each well. The cells were, thereafter, frozen and thawed repeatedly, and then, all cell lysates were collected. According to the BCA total protein assay kit (Nanjing Jiancheng Bioengineering Institute) instructions, the protein content was detected by measuring the absorbance, and the protein concentration was calculated according to that the absorbance is proportional to the concentration.

Statistical analysis

All processed data were expressed in a form of mean ± standard deviation. The difference analysis was performed by SPSS 18.0 software (IBM, Ammon, New York, USA) for statistical analysis (t-test). The $P < 0.05$ indicates a significant difference while $P < 0.01$ indicates a very significant difference.

Results

HPLC-MS/MS detection method of nuciferine

Under the extraction method and detection conditions of this study, the linear relationship was good in the concentration range of 10-5,000 ng/mL. The slope, intercept, and correlation coefficient were calculated by linear regression method. The standard curve equation and correlation coefficient are as follows:

$$Y = 0.0278X + 0.2505 \quad (r=0.9999)$$

Under the conditions of chromatography and mass spectrometry in this study, the average recovery rate and accuracy of nuciferine in cell culture medium were over 97.31% and 95.92%, respectively. The recovery rate and accuracy were 97.31±3.13% and 99.00±6.44% when the concentration of nuciferine was 10 ng/mL, 98.69±1.19% and 99.61±1.34% when the concentration of nuciferine was 100 ng/mL, and 97.81±1.07% and 95.92±3.68% when the concentration of nuciferine was 1,000 ng/mL, respectively. The intra-day and inter-day

variation coefficients were lower than 7.71% with the inter-day and intra-day precisions were 3.22% and 7.71% when the concentration of nuciferine was 10 ng/mL, 1.21% and 7.09% when the concentration of nuciferine was 100 ng/mL, and 1.11% and 6.82% when the concentration of nuciferine was 1,000 ng/mL, respectively. The stability of nuciferine under 4℃ and -20℃ different storage conditions was over 97.78% with 97.92±1.08% and 101.21±3.19% when the concentration of nuciferine was 10 ng/mL, 97.78±0.95% and 100.17±2.46% when the concentration of nuciferine was 100 ng/mL, and 98.91±1.93% and 103.14±5.78% when the concentration of nuciferine was 1,000 ng/mL, respectively. All those data indicate that the quantitative analysis method of nuciferine established in this study can fully meet the research needs.

Detection of cell density

The resistivity of Caco-2 cells on both AP and BL sides showed that the resistivity was 400 Ω/cm^2, which could meet the requirements of drug transport experiment. The activity of alkaline phosphatase on AP side is more than 10 times on BL side, which indicates that cells have polarity. According to the equation of phenol red standard curve,

$$Y = 2.280X + 451.66 \ (R2=0.9993)$$

the corresponding concentration and the cumulative amount in different time periods were calculated. The detection results showed that the transmittance of phenol red in Trans-well chamber was less than 3% within 0-2 hours, which indicated that Caco-2 cells monolayer had good cell density after cultured in the chamber for 21 days, and therefore, could be used for subsequent transmembrane transport experiments.

Trans-membrane transport of nuciferine in Caco-2 cells

The results showed that the apparent permeability coefficients Papp of nuciferine AP→BL and BL→AP at different concentrations were less than 4×10^{-4} cm/s, which indicated that nuciferine had poor permeability in Caco-2 cells. The Papp numbers of high, middle, and low concentrations of nuciferine were significantly different (P < 0.05), and the Papp of high concentration treatment group was significantly lower than that of middle and low concentration treatment groups, which indicated that the transmembrane transport of nuciferine was related to concentration and possibly related to inflow transporters. However, there was no significant difference observed between middle and low concentration treatment groups (P > 0.05).

Comparing to AP→BL, the Papp of BL→AP had no significant difference (P > 0.05) between medium and low nuciferine concentration groups. The similar results were observed between AP→BL and BL→AP in the nuciferine combined verapamil group (P > 0.05), indicating that P- glycoprotein had no significant effect on the efflux of nuciferine (Table 1).

Table 1. Papp (cm × 10^{-6}/s) of nuciferine in Caco-2 monolayer cells at different concentrations (n = 5).

Concentration (ng/mL)	AP→BL	BL→AP
500	3.68 ± 0.74	3.41 ± 0.33
2,500	3.05 ± 0.23	3.30 ± 0.68
10,000	1.27 ± 0.27*	3.32 ± 0.48
nuciferine +verapamil	3.38 ± 0.23	3.17 ± 0.82

*P < 0.05 when comparing to 500 and 2,500 ng/mL groups.

Influencing factors of nuciferine transmembrane transport in Caco-2 cells

The results showed that the transport amount of nuciferine in Caco-2 cells was significantly different at different pH values in 2,500 ng/mL. Comparing to the control group, during 0-1 h, the transport amount of nuciferine in the buffer solution groups of pH 6.0 and 6.5 increased significantly (P < 0.05), while the transport amount of nuciferine in the treatment group of pH 7.0 had no significant difference (P > 0.05), indicating that acidic environment could

promote the transport of nuciferine (Figure 1). The transport capacity of nuciferine in chlorogenic acid and caffeic acid treatment groups were significantly increased comparing to the control group, which indicated that chlorogenic acid and caffeic acid could significantly promote the transmembrane transport of nuciferine (Figure 2).

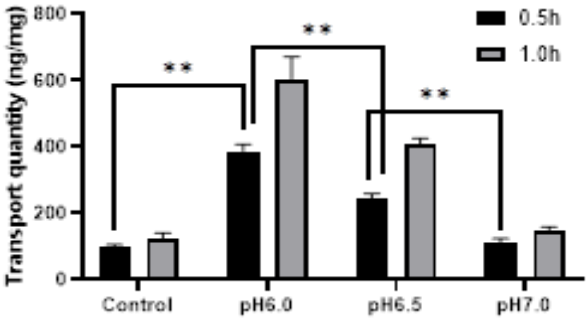

Figure 1. The effects of different pH values on nuciferine transmembrane transport. **P < 0.01.

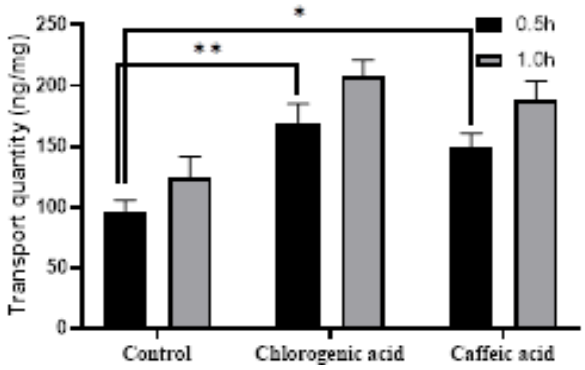

Figure 2. The effects of caffeic acid and chlorogenic acid on nuciferine transmembrane transport. *P < 0.05. **P < 0.01.

In this study, Caco-2 cells were treated with tetraethylamine to investigate the effect of tetraethylamine on the transmembrane transport of nuciferine. The results showed that tetraethylamine could significantly inhibit the transmembrane transport of nuciferine (P < 0.05), which proved that the transmembrane transport of nuciferine might be related to organic cation transporters (Figure 3). As a specific inhibitor of Na^+ pump, ouabain is widely used in cell energy metabolism. In this study, the cells were pretreated with ouabain, and then the influence of Caco-2 cells on the transport of nuciferine was investigated. Comparing to the control group, there was no significant difference in the trans-membrane transport of nuciferine by Caco-2 cells under the intervention of ouabain (Figure 3), which indicated that the trans-membrane transport of nuciferine was mainly passive transport, which facilitated diffusion to a certain extent.

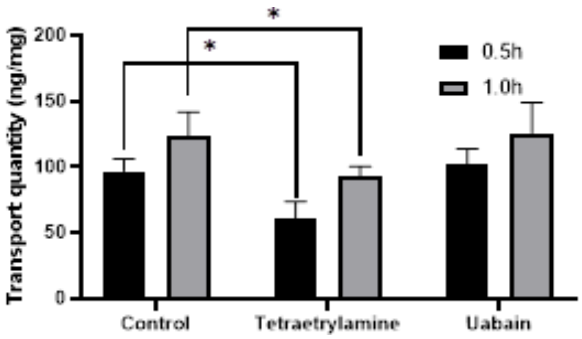

Figure 3. The effects of tetraethylamine and ouabain on nuciferine transmembrane transport. *P < 0.05.

Discussion

In this study, the transport of nuciferine on Caco-2 monolayer cells was studied. The results showed that the transmembrane transport of nuciferine was related to its concentration. After verapamil was added, the Papp of nuciferine did not change significantly, which indicated that inhibition of P-glycoprotein had no significant effect on the transmembrane transport of nuciferine. Comparing the transport amount of nuciferine in Caco-2 cells under different pH conditions, it showed that acidic environment could promote the transport of nuciferine, which might be related to promoting the dissolution of nuciferine. However, the transportation of nuciferine decreased significantly after adding tetraethylamine, which indicated that tetraethylamine had significant inhibitory activity on the transportation of nuciferine. Tetraethylamine, as a specific substrate for

organic cation transporters on the cell surface [18-20] may compete with nuciferine for organic cation transporters on the cell membrane surface, thus reducing the transport capacity of nuciferine, indicating that the transmembrane transport of nuciferine in Caco-2 cells may have both passive diffusion and transporter-mediated inflow transport.

In recent years, it has been reported in literatures that lotus leaf can be made into compound preparation with Alisma orientale, Hawthorn, and Gynostemma pentaphyllum for reducing blood lipid, and there are abundant organic acids in the compound composition [21, 22]. Zheng *et al*. studied the active substance basis of compound lotus leaf capsules from the perspective of serum pharmacology, and the results also confirmed that compound lotus leaf capsules contained active ingredients such as nuciferine, chlorogenic acid, and caffeic acid. According to the principle of Traditional Chinese Medicine composition, lotus leaf and these traditional Chinese medicines may have some synergistic pharmacological effects [23]. Therefore, whether nuciferine and these natural organic acids have synergistic effects in intestinal transport and absorption is worthy further study. This study used both nuciferine and caffeic acid/chlorogenic acid together. The results showed that chlorogenic acid and caffeic acid could significantly promote the transport of nuciferine, which might be helpful to explain the principle of lotus leaf and these traditional Chinese medicines. Ouabain is a typical sodium pump inhibitor, which is related to ATP metabolism. After adding Ouabain, the amount of nuciferine transport did not change significantly, which indicated that the relationship between nuciferine transport and cell energy metabolism was not significant. In addition, this study only explored the transport characteristics and influencing factors of nuciferine at the cellular level. The absorption characteristics and influencing factors of nuciferine at the intestinal level deserve further exploration.

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References

1. Pulok KM, Debajyoti M, Amal KM, SR, Michael H. 2010. The sacred lotus (*Nelumbo nucifera*)– phytochemical and therapeutic profile. J Pharm Pharmacol. 61(4):407-422.
2. Ho HH, Hsu LS, Chan KC, Chen HM, Wu CH, Wang CJ. 2010. Extract from the leaf of nucifera reduced the development of atherosclerosis *via* inhibition of vascular smooth muscle cell proliferation and migration. J Food Chem Toxicol. 48(1):159-168.
3. Ye LH, Kong LT, Yan MZ, Cao FR, Wang LS, Liao YH, *et al*. 2016. Lotus leaf alkaloid fraction can strongly inhibit CYP2D6 isoenzyme activity. J Ethnopharmacol. 194:913-917.
4. Liu F, Zhu R, Lin X, Geng Y, Wang X, Huang L. 2014. Cloud Point Extraction and Pre-Concentration of Four Alkaloids in *Nelumbo nucifera* Leaves by Ultrahigh Pressure-Assisted Extraction. J Sep Sci Technol. 49(7):981-987.
5. Ning Q, Wang Y, Zhang Y, Shen G, Pang J. 2019. Nuciferine prevents hepatic steatosis by regulating lipid metabolism in diabetic rat model. J Open Life Sci. 14(1):699-706.
6. Zhang C, Deng JJ, Liu D, Tuo XX, Xiao L, Lai BC, *et al*. 2018. Nuciferine ameliorates hepatic steatosis in high-fat diet/streptozocin-induced diabetic mice through a PPARα/PPARγ coactivator-1α pathway. Brit J Pharmaco. 175(22):4218-4228.
7. Shu G, Qiu Y, Hao J, Fu Q, Deng X. 2019. Nuciferine alleviates acute alcohol-induced liver injury in mice: Roles of suppressing hepatic oxidative stress and inflammation *via* modulating miR-144/Nrf2/HO-1 cascade. J Funct Foods. 58:105-113.
8. Li Z, Chen Y, An T, Liu P, Zhu J, Yang H, *et al*. 2019. Nuciferine inhibits the progression of glioblastoma by suppressing the SOX2-AKT/STAT3-Slug signaling pathway. J Exp Clin Canc Res. 38(1):139.
9. Xu J, Ying A, Shi T. 2020. Nuciferine Inhibits Skin Cutaneous Melanoma Cell Growth by Suppressing TLR4/NF-κB Signaling. Anti-Cancer Agent Me. 20(17):2099-2105.
10. Nakamura S, Nakashima S, Tanabe G, Oda Y, Yokota N, Fujimoto K, *et al*. 2013. Alkaloid constituents from flower buds and leaves of sacred lotus (*Nelumbo nucifera*, *Nymphaeaceae*) with melanogenesis inhibitory activity in B16 melanoma cells. Biooragn Med Chem. 21(3): 779-787.
11. Wang FG, Cao J, Hou XQ, Li ZY, Qu XL. 2018. Pharmacokinetics, tissue distribution, bioavailability, and excretion of nuciferine, an alkaloid from lotus, in rats by LC/MS/MS. Drug De Ind Pharm. 44(9):1557-1562.

12. Xu YY, Bao SH, Tian WQ, Wen CC, Hu LF, Lin CL. 2015. Tissue distribution model and pharmacokinetics of nuciferine based on UPLC-MS/MS and BP-ANN. Int J Clin Exp Med. 8(10):17612.
13. Guo FC, Xue Y, Li XX, Feng RN, Guan CM, Wang YW, *et al*. 2013. Nuciferine Prevents Hepatic Steatosis and Injury Induced by a High-Fat Diet in Hamsters. PLoS ONE. 8(5):e63770.
14. Wu XL, Wu MJ, Chen XZ, Ma HL, Ding LQ, Qiu F, *et al*. 2017. Metabolic profiling of nuciferine in rat urine, plasma, bile and feces after oral administration using ultra-high performance liquid chromatography-diode array detection-quadrupole time-of-flight mass spectrometry. J Pharm Biomed Anal. 140:71-80.
15. Ye LH, Xiao BX, Liao YH, Liu XM, Pan RL, Chang QI. 2016. Metabolism profiles of nuciferine in rats using ultrafast liquid chromatography with tandem mass spectrometry. Biomed Chromatogr. 30(8):1216-1222.
16. Shi Z, Fang Z, Gao X, Yu H, Liu G. 2021. Nuciferine improves high-fat diet-induced obesity by reducing intestinal permeability through increasing autophagy and remodeling the gut microbiota. Food Funct. 12(13):5850-5861.
17. Zhang C, Deng JJ, Liu D, Tuo XX, Yu Y, Yang HX, *et al*. 2018. Nuciferine Inhibits Proinflammatory Cytokines *via* the PPARs in LPS-Induced RAW264.7 Cells. Molecules. 23(10):2723.
18. Nirmal J, Velpandian T, Singh SB, Biswas NR, Thavaraj V, Azad R, *et al*. 2011. Development and validation of a highly sensitive LC–MS/MS method for organic cation transporter (OCT) substrate tetraethylammonium (TEA) in rabbits. J Chromatography B. 879(9-10):585-590.
19. Quinones AL, Salvador-Vieira L, Wang J. 2021. Organic Cation Transporter 3 Mediates Tissue Uptake and Accumulation of meta-iodobenzylguanidine (mIBG). FASEB J. 35(S1).
20. Li ZH, Ma PK, Huang YF, Zhang Z, Zheng W, Chen JH. 2021. Jiaotai Pill () Alleviates Insomnia through Regulating Monoamine and Organic Cation Transporters in Rats. Chin J Integr Med. 27(3):183-191.
21. Wang Z, Zhang R, Song Q, Zhang XW, Zhang YM. 2015. Effect of compound preparation of lotus leaf, Gynostemma pentaphyllum and perilla leaf extracts on reducing weight and lipid [J]. Chin J Exp Tradit Med Form. 21(13):144-147.
22. Xiao CX, Yang MT, Chen ZJ, Zhou BJ. 2018. Study on quality control of liver protection and remove the fat granules [J]. Chin Materia Med. 041(006):1408-1414.
23. Zheng S, Lang YY, Wang GY, Wen HY, Wang Q. 2017. Preliminary study on Serum Pharmacochemistry of compound lotus leaf Capsule. Chin J Hospital Phar. 37 (011):1056-1059.