Saudi Pharmaceutical Journal 25 (2017) 633–637

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Study on apoptosis effect of human breast cancer cell MCF-7 induced by lycorine hydrochloride via death receptor pathway

Yubin Ji [a,b], Miao Yu [a,b,*], Zheng Qi [a,b], Di Cui [a,b], Guosong Xin [a,b], Bing Wang [a,b], Weiling Jia [a], Lin Chang [a]

[a] Research Center on Life Sciences and Environmental Sciences, Harbin University of Commerce, Harbin 150076, China
[b] Engineering Research Center of Natural Anticancer Drugs, Ministry of Education, Harbin 150076, China

ARTICLE INFO

Article history:
Available online 28 April 2017

Keywords:
Lycorine hydrochloride
MCF-7
Death receptor pathway
Apoptosis

ABSTRACT

As research was conducted on the early apoptosis of human breast cancer cell MCF-7 caused by lycorine hydrochloride and the expression of the related apoptosis proteins. The early-period apoptosis rate of human breast cancer cell MCF-7 was tested with the AnnexinV/PI double staining and flow cytometry. The Western Blotting method was also used to detect the protein expression conditions of Fas, FasL, Caspase-8 and Bid. The results showed that the higher the dose, the higher the rate of apoptosis and that the rate of apoptosis was dependent on the dose; the relative protein activity of Fas, FasL, Caspase-8 and bid gradually rose with the increase of lycorine dosage and the activities revealed certain dose-independence. Results showed that lycorine hydrochloride could induce the apoptosis of human breast cancer cell MCF-7 through the death receptor pathway.

1. Introduction

Alkaloid is an important chemical component that has a significant pharmacological activity in Chinese herbal medicine and exists widely in nature. Lycorine is a natural alkaloid extracted from Amaryllidaceae. Lycorine and its derivatives have multiple functions such as removing heat, detoxification, expelling phlegm, and alleviating carbunclers, sore throat, edema actions. (Liu et al., 2011) It is particularly significant in the treating tumors (Liu et al., 2016; Iftakhar et al., 2015; Liu, 2012; Evidente et al., 2009). Death receptor is one of the main pathways that induce cell apoptosis. Eight members of the death receptor family have been characterized so far, which mainly consist of three types of signal transduction pathways, i.e., TNFR, TRAIL and Fas/FasL signaling pathways. This article will focus on Fas/FasL pathway. In this paper, we have research on the early apoptosis of human breast cancer cells MCF-7 caused by lycorine hydrochloride and the expression of the related apoptosis proteins, in order to investigate the apoptotic pathways (Chen et al., 2016; Wylli, 2010; Rcuhold and Eschcnhurg, 2012).

2. Material and methods

2.1. Cell

MCF-7 cell line was provided by the Life Science and Environmental Science Research Center of Harbin University of Commerce.

2.2. Reagents and drugs

Lycorine Hydrochloride (mass fraction 98%), Aladdin reagent company; Hydroxy camptothecin (mass fraction 98%), Harbin Medisan Pharmaceutical Co., Ltd.; Fetal bovine serum, Hangzhou Sijiqing Biological Engineering Company; RPMI-1640, American GIBCO company; AnnexinV-FITC apoptosis detection kit, USA BD Biological Technology Co., Ltd.; Goat anti mouse lgG horseradish peroxidase labeling, Beijing Zhongshan Golden Bridge Biotechnology Co., LTD; Mouse anti-actin monoclonal antibody, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.

2.3. Instruments

DL-CJ-1N type super clean workbench, Beijing donglianha'er Instruments Manufacturing Co., LTD; Adventurer electronic balance, OHAUS Company; CO-150 type carbon dioxide cell incubator,

* Corresponding author at: Research Center on Life Sciences and Environmental Sciences, Harbin University of Commerce, Harbin 150076, China.
E-mail address: yumiao913@163.com (M. Yu).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2017.04.036
1319-0164/

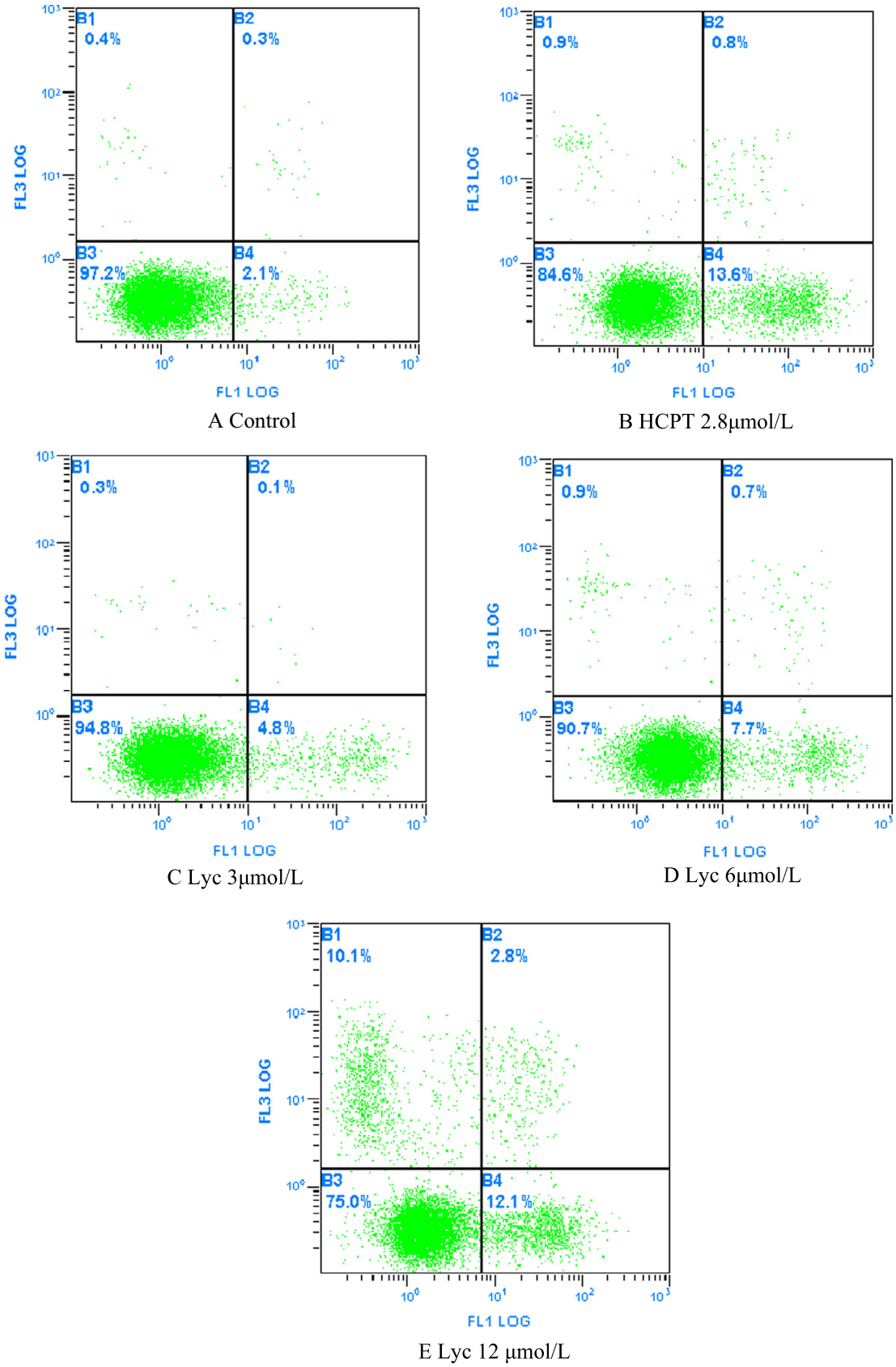

Fig. 1. The detection of the early apoptosis rate of MCF-7 cells using low cytometry.

the United States New Blanche Vick Science Company; CKX-41-32 type inverted microscope, Japan OLYMPUS company; EPICS-XL flow cytometry, the United States Coulter Beckman company; P-type micro displacement device, Gilson company; DYY-7C type electrophoresis instrument, 61 instrument factory; AllegraX-5 low temperature high speed centrifuge, Beckman-Coulter Limited

by Share Ltd; JY-SCZ3 type vertical electrophoresis transfer tank, 61 instrument factory; TL-2000MM-III type multi speed oscillator, Jiangyan Tianli Medical Instrument Company; Gel imaging system GIS-2019, Tannon Company.

2.4. Cell culturing and passage

MCF-7 cells were cultured in RPMI-1640 medium supplemented with 12% fetal bovine serum and incubated at 37 °C in 5% CO_2 saturated humidity. When the cells reached a high density of about 80%, the cells could pass through with the logarithmic growth phase of the cells to experiment.

2.5. Apoptosis assays

The MCF-7 cells inoculated in the logarithmic growth phase were into a 6-well microtiter plates at 1.0×10^5 cells/1-mm-diameter well. Lycorine solution and HCPT solution was added after 24 h. The final concentration of lycorine in the experimental group was 3, 6 and 12 μmol/L, the final concentration of HCPT group was 2.8 μmol/L, and the control group was added with the equal amounts of RPMI-1640 medium. After being cultured for 48 h, in a 5% CO_2 incubator at 37 °C, the MCF-7 cells were collected in the centrifuge tubes and centrifuged at 1500 rpm for 5 min. After that, the supernatant was discarded. Then, the cells were resuspended in PBS and the cells were resuspended in 100,000. The cells were centrifuged at 1500 rpm for 5 min. The supernatant was discarded and 100 μL of the diluted 1× conjugate was pipetted. 5 μL of PI and Annexin V-FITC staining were added respectively, cells were mixed at room temperature away from light for 2 min, then 300 μL of diluted 1× liquid was added, and cells were mixedin the dark place with ice bath. Cell suspension were filtered with 300 mesh nylon mesh before detection. After detection, the testing was under 488 nm excitation wavelength and 525 nm emission wavelength was (Tao et al., 2016; Safi et al., 2015; Bebenek et al., 2007).

2.6. Protein extraction and western blotting

The MCF-7 cells in the logarithmic growth phase were inoculated into the large culture bottle at 2.5×10^6 cells/mL. The final concentration of lycorine in the experimental group was 3, 6 and 12 μmol/L, and the final concentration of HCPT group was 2.8 μmol/L. The control group was added with the same amount of RPMI-1640 medium. After incubation for 48 h at 37 °C in a 5% CO_2 incubator, all the protein of MCF-7 cells incubated for 48 h was extracted by centrifugation at 15,000 r/min for 15 min and 4 °C, the protein content was detected by BCA Protein Concentration Assay Kit. After 10 min of denaturation at 100 °C for 10 min, 20 μL of 5× protein was added to each well, electrophoresed in 12% SDS - PAGE gel, 80 V to the bottom, and transferred to nitrocellulose membrane. Blots were blocked with 5% non-fat dry milk for more than 1 h prior to primary antibody was incubated with at 4 °C overnight. After washing the membrane and incubation with secondary antibodies for 2 h at room temperature, the bands of interest were revealed by DAB and then photographed and analyzed with a gel imaging system.

2.7. Statistical analysis

Statistical analysis was using SPSS 17.0 software. The results were expressed as mean ± standard deviation, and *t*-test used to compare the two sample means. There was a statistically significant as $P < 0.05$.

3. Results

3.1. Using Annexin V-FITC/PI double staining to detect early apoptosis of cells

The flow cytometry showed that Lycorine acted on human MCF-7 cells apoptosis induction (Fig. 1). Cells in the first quadrant were necrotic and had late apoptotic cells that could be simultaneously stained with Annexin V-FITC /PI. The second quadrant cells were propidium iodide-negative cells. In the third quadrant, normal

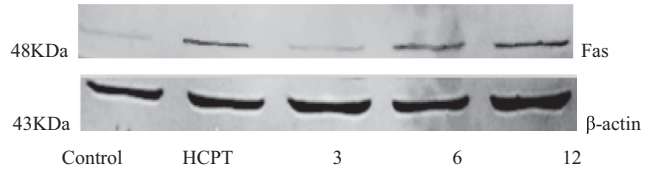

Fig. 2. Effect of lycorine hydrochloride on the expression of Fas protein in MCF-7 cells.

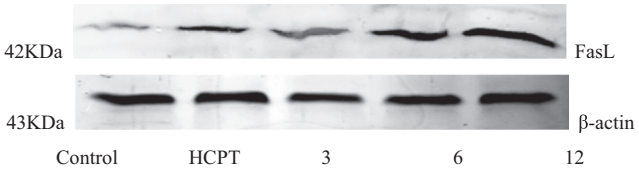

Fig. 4. Effect of lycorine hydrochloride on the expression of FasL protein in MCF-7 cells.

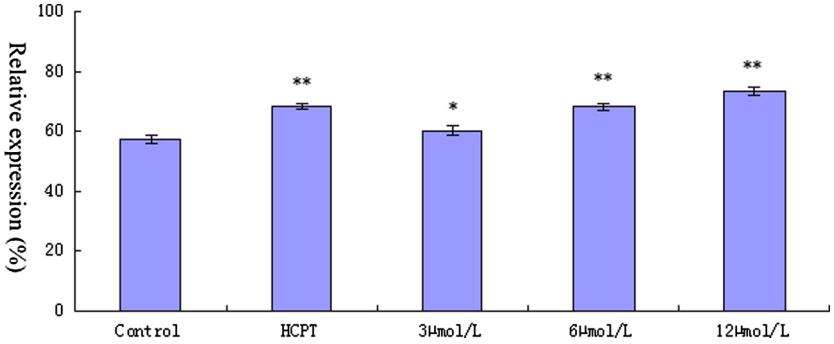

Fig. 3. Effect of lycorine hydrochloride on the expression of Fas protein in MCF-7 cells, compared with the negative control group: $^{*}P < 0.05$, $^{**}P < 0.01$.

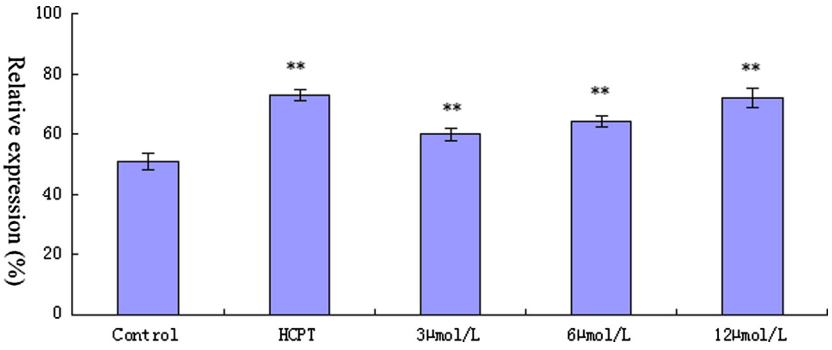

Fig. 5. Effect of lycorine hydrochloride on the expression of FasL protein in MCF-7 cells, compared with the negative control group: $^{*}P < 0.05$, $^{**}P < 0.01$.

living cells were not stained with Annexin V-FITC and propidium iodide (PI). The cells in the fourth quadrant were only stained by Annexin V-FITC for early apoptotic cells. The early apoptotic rate of the drug-treated group were (4.67 ± 0.32) %, (7.63 ± 1.10) % and (11.43 ± 1.88) % respectively, and the early poptotic rate of the positive group was (2.03 ± 0.43) %, which was statistically significant ($P < 0.01$) between positive group and negative control group . Thus, it could be concluded that as the dose increased, so did the apoptosis rate.

3.2. The detection of the expressions of Fas, FasL, Caspase-8 and Bid by western-blot

3.2.1. The detection of expressions of Fas using western-blot

After the MCF-7 cells were treated with Lycorine (3, 6, 12 μmol/L) for 48 h, the western-blot analysis result showed that the gray value of the Fas protein gradually increased as the dosage increased. The results showed that lycorine could up-regulate the expression of Fas protein. It was statistically significant ($P < 0.05$) between Fas group and negative control group. The experimental results are shown in Figs. 2 and 3.

3.2.2. The detection of the expressions of FasL by western-blot

After the MCF-7 cells were treated with Lycorine (3, 6, 12 μmol/L) for 48 h, the western-blot analysis result was gray value of FasL protein gradually increased as the dosage increased. The results showed that lycorine could up-regulate the expression of FasL protein. It was statistically significant ($P < 0.05$) between FasL protein expression and negative control group. The experimental results are shown in Figs. 4 and 5.

3.2.3. The dectection of the expressions of Caspase-8 by western-blot

After the MCF-7 cells were treated with Lycorine (3, 6, 12 μmol/L) for 48 h, the western-blot analysis result showed that the gray value of Caspase-8 protein gradually increased as the dosage increased. lycorine could up-regulate Caspase-8 protein expression. It was statistically significant ($P < 0.05$) between Caspase-8 protein expression and negative control group. The experimental results are shown in Figs. 6 and 7.

3.2.4. The detection of expressions of BID by western-blot

After the MCF-7 cells were treated with Lycorine (3, 6, 12 μmol/L) for 48 h, the western-blot analysis showed the gray value of BID protein gradually increased as the dosage increased. The results

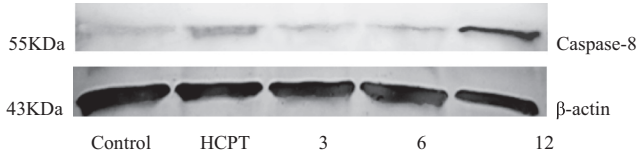

Fig. 6. Effect of lycorine hydrochloride on Caspase-8 protein expression in MCF-7 cells.

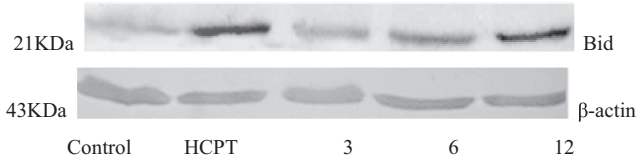

Fig. 8. Effect of lycorine hydrochloride on the expression of Bid protein in MCF-7 cells.

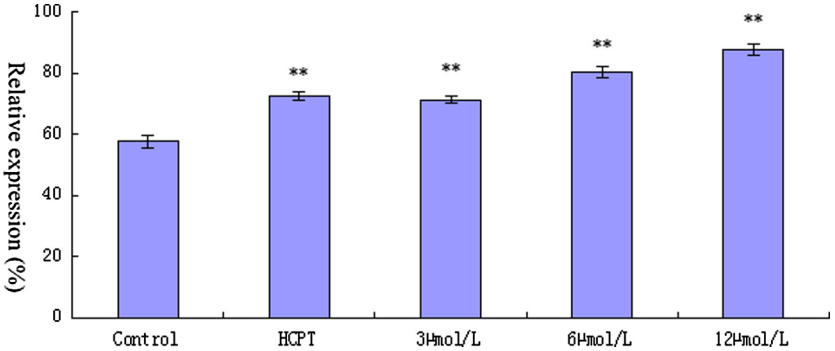

Fig. 7. Effect of lycorine hydrochloride on the expression of Caspase-8 protein in MCF-7 cells, compared with the negative control group: $^{*}P < 0.05$, $^{**}P < 0.01$.

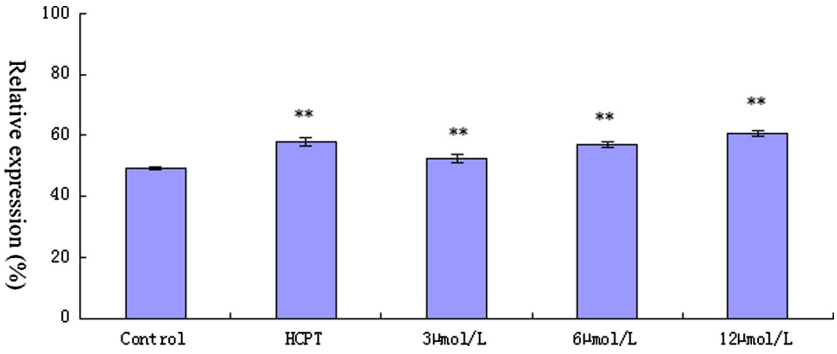

Fig. 9. Effect of lycorine hydrochloride on the expression of Bid protein in MCF-7 cells, compared with the negative control group: $^{*}P < 0.05$, $^{**}P < 0.01$.

showed that lycorine could up-regulate the expression of BID protein. It was statistically significant ($P < 0.05$) between BID protein expression and negative control group. The experimental results are shown in Figs. 8 and 9.

4. Discussion

After treating MCF-7 cells with lycorine, and Annexin V-FITC/PI kit test results demonstrated that lycorine could inhibit the growth of MCF-7 cells, and the effect was relatively obvious. The inhibitory rate was dose-dependent, and the inhibition rate increased with the increase of drug dosage (Feng et al., 2016). Thus, it was concluded that lycorine could induce the early apoptosis of the MCF-7 cells.

The western-blot analysis detected the expression of Fas, FasL, Caspase-8 and BID protein, because Fas, FasL, Caspase-8 and BID proteins are the key proteins in the death receptor pathway of cell apoptotic. The above proteins' expression showed the cells were inducedapoptosis of cancer cells. According to current work findings, lycorine can induce MCF-7 cell apoptosis. The mechanism may be through the up-regulation of Fas/FasL protein expression, thereby promoting the expression of Caspase-8 protein, and activating the downstream Caspase-3, thus inducing a series of related cascaded reactions and triggering apoptosis (Xie et al., 2016; Yine et al., 2015; Lamoral-Theys et al., 2009). It was demonstrated that lycorine could induce MCF-7 cell apoptosis through the death receptor pathway. Many research before promoted the up-regulation of the expression of Bid, it both links the death receptor pathway and mitochondrial pathway, which through the synergistic effect to jointly induce the apoptosis of MCF-7 cells.

5. Conclusions

The results showed that the higher the dosage, the higher the rate of MCF-7 cells' apoptosis, and the rate of apoptosis was dependent on the dosage; the relative protein activity of Fas, FasL, Caspase-8 and bid gradually increased with the increase of lycorine dosage, presenting certain dose-independence. Results also showed that lycorine hydrochloride could induce apoptosis of MCF-7 cells through the death receptor pathway.

Acknowledgements

This work is supported by the Postdoctoral Foundation of China (Grant No. 2015M581467), the Natural Science Foundation of Heilongjiang Province (Grant No. D201138), the Postdoctoral Foundation of Hei Long Jiang Province (Grant No. LBH-Z15107), the Science and Technology Innovation Team Program in Higher Education Institutions of Heilongjiang Province (Grant No. 2014TD009), the Harbin Special Foundation for Young Technological Innovative Talented Person (Grant No. 2013RFQXJ150) and the Training Program for Young Innovative Talented Person of Heilongjiang Province (Grant No. UNPYSCT-2015071).

References

Bebenek, M., Duś, D., Koźlak, J., 2007. Fas/Fas-ligand expressions in primary breast cancer are significant predictors of its skeletal spread. J. Anticancer Res. 27, 215–218.
Chen, Y., Gao, Y., Ashraf, M.A., Gao, W., 2016. Effects of the traditional chinese medicine dilong on airway remodeling in rats with OVA-induced-Asthma. Open Life Sci. 11 (1), 498–505.
Evidente, A., Kireev, A.S., Jenkins, A.R., et al., 2009. Biological evaluation of structurally diverse amaryllidaceae alkaloids and their synthetic derivatives: discovery of novel leads for anticancer drug design. J. Planta Med. 75, 501–507.
Feng, B., Ashraf, M.A., Peng, L., 2016. Characterization of particle shape, zeta potential, loading efficiency and outdoor stability for chitosan-ricinoleic acid loaded with rotenone. Open Life Sci. 11 (1), 380–386.
Iftakhar, A., Hasan, I.J., Sarfraz, M., Jafri, L., Ashraf, M.A., 2015. Nephroprotective effect of the leaves of Aloe barbadensis (Aloe Vera) against toxicity induced by diclofenac sodium in albino rabbits. West Indian Med. J. 64 (5), 462–467.
Lamoral-Theys, D., Andolfi, A., Goietsenoven, G.V., et al., 2009. Lycorine, the main phenanthridine Amaryllidaceae alkaloid, exhibits significant anti-tumor activity in cancer cells that display resistance to proapoptotic stimuli: an investigation of structure-activity relationship and mechanistic insight. J. Med. Chem. 52, 6244–6256.
Liu, J., Yang, Y., Xu, Y., et al., 2011. Lycorine reduces mortality of human enterovirus 71-infected mice by inhibiting virus replication. Virol. J. 8, 483–492.
Liu, Z.L., 2012. The dynamic analysis of China's energy-economy-environment system: VAR and VEC modeling. Adv. Inform. Sci. Service Sci. 4 (14), 210–218.
Liu, Z.K., Gao, P., Ashraf, M.A., Wen, J.B., 2016. The complete mitochondrial genomes of two weevils, Eucryptorrhynchus chinensis and *E. brandti*: conserved genome arrangement in Curculionidae and deficiency of tRNA-Ile gene. Open. Life Sci. 11 (1), 458–469.
Rcuhold, T.F., Eschcnhurg, S., 2012. A molecular view on signal transduction by the apoptosome. J. Cell Signal. 24, 1420–1425.
Safi, S.Z., Qvist, R., Chinna, K., Ashraf, M.A., Paramasivam, D., Ismail, I.S., 2015. Gene expression profiling of the peripheral blood mononuclear cells of offspring of one type 2 diabetic parent. Int. J. Diabetes Dev. Countries 2015, 1–8.
Tao, X., Ashraf, M.A., Zhao, Y., 2016. Paired observation on light-cured composite resin and nano-composite resin in dental caries repair. Pak. J. Pharm. Sci. 29 (6), 2169–2172.
Wylli, A.H., 2010. "Where, O death, is thy sting?" A brief review of apoptosis biology. J. Mol. Neurobiol. 42, 4–9.
Xie, H., Huang, H., He, W., Fu, Z., Luo, C., Ashraf, M.A., 2016. Research on in vitro release of Isoniazid (INH) super paramagnetic microspheres in different magnetic fields. Pak. J. Pharm. Sci. 29 (6), 2207–2212.
Yine, H., Shufang, D., Bin, W., Wei, Q., Junling, G., Ashraf, M.A., 2015. Analysis of the relations between allergen specific LgG antibody and allergic dermatosis of 14 kinds foods. Open Med. 10, 405–409.