doi.org/10.36721/PJPS.2020.33.3.SP.1383-1388.1

Effect of hesperidin on CORT-induced apoptosis and oxidative stress of mouse hippocampal nerve cells by up-regulating miR-146a-5p

Zhiheng Wang[1], Lijun Han[2]*, Huizi ShangTing[3], Ping Chen[4]
[1]Xinhua Community Health Service Center in Wulong Street, Qiqihar, China
[2]Pathology School, Qiqihar Medical University, Qiqihar, China
[3]Graduate School, Qiqihar Medical University, Qiqihar, China
[4]College of Mental Health, Qiqihar Medical University, Qiqihar, China

Abstract: This study aims to investigate the effect of hesperidin on CORT-induced apoptosis and oxidative stress of mouse hippocampal nerve cells by up-regulating miR-146a-5p and related mechanism. Hesperidin was applied to CORT-induced HT-22 cells, or HT-22 cells whose expression of mir-146a-5p was up-regulated or down-regulated by CORT. The apoptosis rate was detected by flow cytometry. Expression of Cleaved-caspase-3 protein in cells was detected by Western blot. The levels of MDA, SOD and CAT in the cells were detected by enzyme-linked immunosorbent assay, and the expression of miR-146a-5p was detected by RT-qPCR. The application of hesperidin or up-regulation of miR-146a-5p can reduce the CORT-induced apoptosis rate of HT-22 cells, Cleaved caspase-3 protein expression and MAD content ($p<0.05$), and increase the activity of SOD and CAT and the expression of miR-146a-5p ($p<0.05$). In contrast, down-regulation ofmiR-146a-5p can increase the CORT-induced apoptosis rate of HT-22 cells, Cleaved caspase-3 protein expression and MAD content ($p<0.05$), and decrease the activity of SOD and CAT and the expression of miR-146a-5p ($p<0.05$). Down-regulation of miR-146a-5p expression can reverse the effects of hesperidin on CORT-induced HT-22 cell apoptosis and oxidative stress. Hesperidin may protect cells from being damaged by up-regulating miR-146a-5p to reduce CORT-induced HT-22 cell apoptosis and oxidative stress.

Keywords: Hesperidin, miR-146a-5p, hippocampal nerve cells, apoptosis, oxidative stress.

INTRODUCTION

Depression is a common chronic neurodegenerative disease, which is often manifested as depression, retardation of thinking and lack of initiative (Lei *et al*., 2018). If the depression is not treated in time, it will bring heavy burden to the patient's family and even affect the patient's life. The increase of cortisol level caused by endocrine system disorder is an important pathological feature of patients with depression (Freitas *et al*., 2015). Therefore, protecting nerve cells from injury is of great significance in the treatment of depression. As a natural monomer extracted from orange peel, Hesperidin has a variety of pharmacological activities such as antioxidant, anti-inflammatory and immune regulation (Cao *et al*., 2018; Kamboh and Zhu, 2014; Tejada *et al*., 2018). Studies have shown that hesperidin reduces the neurotoxicity of rotenone to dopamine neurons (Xu *et al*., 2019). Hesperidin may exert a neuroprotective effect on model rats with 3-nitropropionic acid-induced huntington's disease (Menze *et al*., 2012). It is unclear whether hesperidin can be used to treat depression. microRNAs (miRNA) are a class of small non-coding single-stranded RNSs participating in the regulation of cell proliferation, apoptosis, oxidative stress and other life processes, which play a role in the occurrence and development of neurodegenerative diseases (Wang *et al*., 2018; Wu *et al*., 2017). He *et al*. (He *et al*., 2018) showed that, by up-regulating the expression of miR-146a-5p and down-regulating the expression of GPR17, BaoZhu pill could inhibit the apoptosis of nerve cells in rats with acute spinal cord injury and promote the recovery of spinal cord function, but whether miR-146a-5p was involved in the development of depression remained unknown. Corticosterone (CORT) is a glucocorticoid, the elevated expression of which can lead to neuronal damage and depression-like behaviors (He *et al*., 2017). The research investigates the effect of hesperidin on HT-22 cell apoptosis and oxidative stress and whether it could play a role in regulating the expression of miR-146a-5, providing theoretical basis for the development of drugs to treat depression.

MATERIALS AND METHODS

Cells and laboratory reagents

Ht-22, a mouse hippocampal nerve cell, was purchased from Shanghai Ran Biotechnology Co., Ltd.; hesperidin was purchased from National Institute for the Control of Pharmaceutical and Biological Products. Fetal bovine serum (FBS), DMEM medium, Annexin V-FITC/Propidium Iodide (PI) apoptosis kit and bicinchoninicacid (BCA) protein detection kit were purchased from Beijing Solarbio Science & Technology Co., Ltd. Trypsin was purchased from Sigma, USA; Trizol reagent, reverse transcription kit and PCR kit were

**Corresponding author:* e-mail: hanlijun0325@163.com

purchased from Shenzhen Jingmei Biological Engineering Co., Ltd. PCR primers were designed and synthesized by Shanghai Sangon bioengineering Co., Ltd. LipofectamineTM 2000 kit was purchased from Invitrogen, USA. Rabbit anti-mouse activated caspase-3 (Cleaved caspase-3) polyclonal antibody was purchased from Wuhan Boster Biotechnology Co., Ltd. miR-146a-5p mimcs and mim-negative sequences (miR-NC), mir-146a-5p inhibitors (anti-miR-146a-5p) and inhibitor negative sequences (anti-miR-NC) were all purchased by Shanghai GenePharma Co.,Ltd. Malondialdehyde (MDA) levels, superoxide dismutase (SOD) and catalase (CAT) kits were all purchased from Nanjing Jiancheng Bioengineering Institute.

Experimental method

(1) Cell culture and transfection. The resuscitated HT-22 cells were added to a DMEM culture containing 10% FBS, cultured in a 25 cm2 culture flask, and then transferred to a 37℃ incubator with CO2 volume fraction of 5% under humidity of 97%. The medium was refreshed every 2 days. When the cells were fused to 80%-90%, the culture medium was absorbed, 0.25 % trypsin was digested, and continuous cell culture was conducted in a ratio of 1:3. Ht-22 cells in logarithmic growth stage were inoculated into 6-well plates with 1×105 cells per well. After cell fusion to 60%, miR-146a-5p, miR-NC, anti-miR-146a-5p and anti-miR-NC were transfected into HT-22 cells according to the operation instructions of LipofectamineTM 2000 kit. After transfection for 12 h, fresh medium was replaced for another 48 h of culturing, and trypsin digestion was performed before collecting cells for subsequent experiments.

(2) Cell grouping. Ht-22 cells and cells in the transfected groups were inoculated into 24-well plates, with 2.5×104 cells per well. Ht-22 cells were divided into NC group, CORT group, hesperidin group and hesperidin +CORT group. NC group was applied with normal culture medium. CORT group was treated with medium containing 10 μmol/L (Sun *et al*., 2017) CORT for 24h. Hesperidin group was treated with medium containing 100 μmol/L (Xu *et al*., 2019) hesperidin for 24h. Hesperidin+CORT group was treated medium containing 10 μmol/L CORT and 100 μmol/L hesperidin for 24 h. HT-22 cells transfected with miR-146a-5p, miR-NC, anti-miR-146a-5p and anti-miR-NC were all treated with medium containing 10 μmol/L CORT for 24 h, which are denoted as CORT+ miR-146a-5p group, CORT+ miR-NC group, CORT+ anti-miR-146a-5p group and CORT+ anti-miR-NC group, respectively. Ht-22 cells transfected with anti-miR-146a-5p and anti-miR-NC were treated with medium containing 10 μmol/L CORT and 100 μmol/L hesperidin for 24 h, which are denoted as CORT+ hesperidin + anti-miR-146a-5p group and CORT+ hesperidin + anti-miR-NC group, respectively. Each group was provided with 3 duplicate holes.

(3) The cells were digested by trypsin and then collected according to section 1.2.2. 1.0×106 cells were washed twice with an appropriate amount of phosphate buffered saline (PBS), and centrifuged at 1500 r/min for 5 min, before sucking PBS out. According to the instructions of Annexin V-FITC/PI kit, 500 μL of binding buffer was added to gently blow the suspension cells, and then 10 μL of Annexin V-FITC was added for Vortex mixing and incubating at room temperature in dark for 10 min. After that, the solution system was added with 5 μL of PI, subjected to vortex blending, and incubated at room temperature in dark for 5 min for detecting cell apoptosis by upflow cytometry.

(4) Detection of cleaved caspase-3 protein expression by Western blot. The cells were digested by trypsin and collected after grouping according to section "(2) Cell grouping". Total protein in cells was extracted from IPA protein lysate, and the protein concentration was determined by BCA method and then quantified. After that, take an appropriate amount of protein solution into the EP tube, add the loading buffer, scroll and mix well, and boil at 100℃ for 5 min. After denaturation of the protein, sodium dodecyl sulfonate-polyacrylamide gel electrophoresis was conducted at a rate of 30 μg protein per well, and then the protein was transferred to a polyvinylidene difluoride film with a rotating film analyzer and sealed in 5% skim milk powder for 2 h. After membrane washing, Cleaved-caspase-3 and β-actin were added, respectively, and incubated overnight at 4℃. After that, membrane was washed again, and horseradish peroxidase labeled secondary antibody was added and incubated at 37℃ for 1 h. Then, membrane was washed once again, followed by addition of ELC developer for development without light, and the gel imaging system was exposed and photographed. With β-actin as internal parameter, Image J was used to analyze the gray value of target protein bands.

(5) The cells were digested by trypsin and collected according to section "(2) Cell grouping". The content of MDA and activities of SOD and CAT in cells were detected by referring to the instructions of MDA, SOD and CAT kit.

(6) Detection of the expression of miR-146a-5p in the cells by real-time fluorescence quantitative PCR (RT-qPCR). Trizol reagent was used to extract total RNA from cells for each group, and the purity and concentration of RNA were determined by micronucleic acid analyzer. After quantification, RNA was reversely transcribed into cDNA according to the instructions of the reverse transcription kit. Finally, cDNA was used as template for amplification according to following procedures: Predenaturation at 95°C for 5 min, denaturation at 95°C for 10 s, annealing at 60°C for 30 s, extension at 72°C for 30 s. A total of 35 cycles were performed. Primer sequence miR-146a-5p upstream 5'-TGAGAACTGAATTCCATGGTT-3',

downstream 5'-GTCCGTGAAGT CGATGCTAAACGT-3'; U6 upstream 5'-CTCGCTTCGGCAGCACA-3', downstream 5'-AACGCTTCACG AATTT GCGT-3'. The relative expression level of miR-146a-5p was calculated using U6 as internal parameter using $2^{-\triangle\triangle Ct}$.

STATISTICAL ANALYSIS

SPSS.22.0 software was used to analyze the experimental data. The measurement data were expressed as mean ± standard deviation ($\bar{x}\pm s$). The independent sample t test was used for the comparison between the two groups; One-way ANOVA was used for comparison among multiple groups, and SNK-q test was used for further pairwise comparison. The difference was considered statistically significant when $p<0.05$

RESULTS

Effect of hesperidin on CORT-induced apoptosis of HT-22 cell

As shown in fig. 1 and table 1, compared with NC group, HT-22 cell apoptosis rate and Cleaved caspase-3 protein expression level were increased in CORT group ($p<0.05$), while HT-22 cell apoptosis rate and Cleaved caspase-3 protein expression level were not significantly changed in hesperidin group ($p>0.05$). Compared with the CORT group, HT-22 cell apoptosis rate and Cleaved caspase-3 protein expression level in the hesperidin +CORT group were decreased ($p<0.05$).

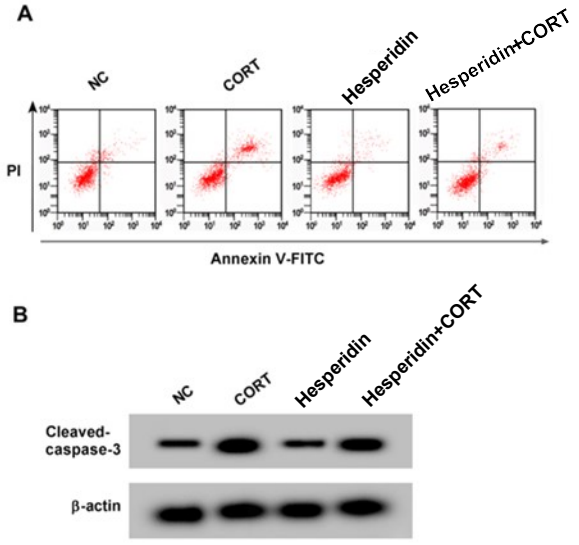

Fig. 1: Effect of hesperidin on CORT-induced apoptosis of HT-22 cell (A: Measuring the effect of hesperidin on CORT-induced apoptosis of HT-22 cell by flow cytometry; B: Effect of hesperidin on Cleaved-caspase-3 protein expression level in CORT-induced HT-22 cell)

Table 1: Effect of hesperidin on CORT-induced apoptosis of HT-22 cell ($\bar{x}\pm s$, n=9)

Group	Cleaved-caspase-3	Apoptosis rate (%)
NC	0.45±0.05	6.35±0.64
CORT	0.96±0.10*	27.55±2.76*
Hesperidin	0.43±0.04	6.45±0.65
Hesperidin +CORT	0.60±0.06#	12.15±1.21#
F	122.441	362.510
p	0.000	0.000

Note: Compared NC group, *p<0.05; Compared with CORT group, #p<0.05.

Effects of hesperidin on MDA, SOD and LDH levels in CORT-induced HT-22 cells

As shown in table 2, compared with NC group, MAD content in HT-22 cells was increased ($p<0.05$) and the activities of SOD and CAT were decreased ($p<0.05$) in CORT group, while MAD content, activities of SOD and CAT in HT-22 cells in hesperidin group remained nearly unchanged ($p>0.05$). Compared with the CORT group, the content of MAD in HT-22 cells was decreased ($p<0.05$), while the activities of SOD and CAT were increased in hesperidin +CORT group ($p<0.05$).

Table 2: Effects of hesperidin on MDA and SOD activity levels in CORT-induced HT-22 cells and LDH activity of cell culture supernatant ($\bar{x}\pm s$, n=9)

Group	MAD (μmol/g)	SOD(U/mg)	CAT(U/mg)
NC	20.01±2.01	15.02±1.51	9.68±0.97
CORT	35.22±3.52*	5.76±0.58*	5.27±0.53*
hesperidin	20.34±2.05	15.36±1.54	9.48±0.95
hesperidin +CORT	24.35±2.44#	12.53±1.25#	8.61±0.86#
F	68.374	109.027	52.659
p	0.000	0.000	0.000

Note: Compared NC group, *p<0.05; compared with CORT group, #p<0.05.

Effect of hesperidin on miR-146a-5p expression in in CORT-induced HT-22 cells

As shown in table 3, compared with the NC group, the expression level of miR-146a-5p in HT-22 cells was decreased ($p<0.05$) in the CORT group, while the expression level of miR-146a-5p in HC-22 cells was not significantly changed in the hesperidin group ($p>0.05$). Compared with the CORT group, the expression level of miR-146a-5p in HT-22 cells was increased in hesperidin +CORT group ($p<0.05$).

Table 3: Effect of hesperidin on miR-146a-5p expression in CORT-induced HT-22 cells ($\bar{x}$±s, n=9)

Group	miR-146a-5p
NC	1.00±0.10
CORT	0.37±0.04*

Effect of miR-146a-5p upregulation on apoptosis and oxidative stress of CORT-induced HT-22 cells

As shown in fig. 2 and table 4, compared with the CORT+ miR-NC group, the expression level of miR-146a-5p in HT-22 cells was increased ($p<0.05$), the expression level of Cleaved caspase-3 protein and apoptosis rate of HT-22 cells decreased ($p<0.05$), the content of MAD was decreased ($p<0.05$), and the activities of SOD and CAT were increased in the CORT+ miR-146a-5p group ($p<0.05$).

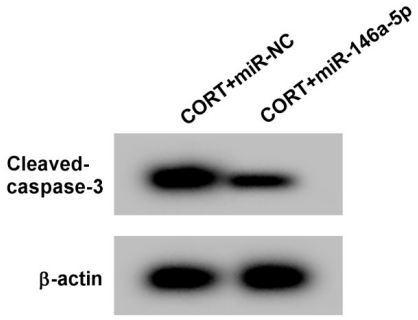

Fig. 2: Effect of miR-146a-5p upregulation on Cleaved-caspase-3 expression level in CORT-induced HT-22 cells.

Effects of mir-146a-5p down-regulation on cort- induced apoptosis rate and oxidative stress of ht-22 cells

As shown in fig. 3 and table 5, compared with the CORT+ anti-miR-NC group, the expression level of miR-146a-5p in HT-22 cells was decreased ($p<0.05$), the expression level of Cleaved caspase-3 protein and apoptosis rate of HT-22 cells was increased ($p<0.05$), the content of MAD increased ($p<0.05$), and the activities of SOD and CAT were decreased in the CORT+ anti-miR-146a-5p group ($p<0.05$).

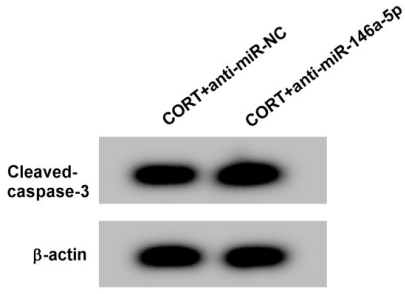

Fig. 3: Effect of miR-146a-5p down-regulation on of Cleaved-caspase-3 expression in CORT-induced HT-22 cells.

As shown in fig. 4 and table 6, compared with the CORT+ hesperidin + anti-miR-NC group, the expression level of miR-146a-5p in HT-22 cells was decreased ($p<0.05$), the expression level of Cleaved caspase-3 protein and apoptosis rate of HT-22 cells were increased ($p<0.05$), the content of MAD was increased ($p<0.05$), and the activities of SOD and CAT were decreased in the CORT+ hesperidin + anti-miR-146a-5p group ($p<0.05$).

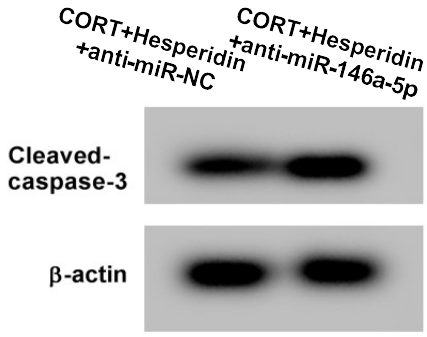

Fig. 4: Downregulation of miR-146a-5p could reverse the effect of hesperidin on Cleaved-caspase-3 expression in CORT-induced HT-22 cells.

DISCUSSION

Depression is a neurodegenerative disease, which affects the physical and mental health of patients and causes great burden to the family of patients and the society. Studies have shown that patients with depression have hippocampal damage, which is mainly manifested as the loss of neurons and decreased hippocampal volume (Zhang *et al*., 2017). Apoptosis of nerve cells is one of the main causes of neuron loss. Inhibiting apoptosis of hippocampal nerve cells and reducing damage to hippocampal tissues is one of the treatment targets of depression (Yang *et al*., 2017). Caspase family belongs to cysteine protease, which plays a key regulatory role in cell apoptosis. Activated caspase-3 can induce apoptosis. This study shows that CORT can promote HT-22 cell apoptosis, while hesperidin may reduce CORT-induced apoptosis of HT-22 cells by inhibiting the expression of activated caspase-3 protein, which may have positive significance for the treatment of depression.

Studies have shown that oxidative stress plays an important role in the occurrence and development of depression. The pathogenic mechanism mainly lies in the excessive generation or reduction of free radicals, which affects biofilm, nucleic acid and cell apoptosis, causing neuronal injury and apoptosis and leading to depression (Zhang *et al*., 2019). MDA is one of the lipid peroxidation products, of which the level can indirectly reflect the level of oxidative stress. SOD is an important antioxidant enzyme in human body, which can remove oxygen free radicals and protect the body from oxidative damage. CAT is also an antioxidant enzyme that rapidly converts hydrogen peroxide into oxygen and water, reducing the

production of hydroxyl free radicals and preventing cell damage and dysfunction caused by oxidative stress. This study showed that after CORT intervention of HT-22 cells, MAD content in the cells was increased and activities of SOD and CAT were decreased, which is consistent with the results of relevant reports, indicating that CORT can induce oxidative stress response in HT-22 cells and cause damage to the cells. However, hesperidin+CORT group exhibited a decreased MAD content in HT-22 cells and increased activities of SOD and CAT, suggesting that hesperidin can reduce CORT-induced oxidative stress response of HT-22 cells and protect the damaged cells.

miRNA is widely found in eukaryotes, which is closely related to the occurrence and development of neurodegenerative diseases. miR-146a-5p is involved in the occurrence and development of a variety of diseases. Studies have shown that miR-146a-5p is down-regulated in non-small-cell lung cancer, liver cancer, breast cancer and other tumors, and up-regulation of miR-146a-5p can inhibit the malignant biological behaviors of tumors and play the role of tumor suppressor genes (Zhang *et al*., 2019; Iacona *et al*., 2019; Yin *et al*., 2019). Studies have also shown that miR-146a-5p is associated with the development of neurological diseases. Xiao Huimei *et al*. showed that miR-146a-5p was highly expressed in serum of children with drug-resistant epilepsy, which can be used for early diagnosis and prognosis evaluation of drug-resistant epilepsy (Xiao *et al*., 2013). Currently, the effect of miR-146a-5p on hippocampal nerve cells remains unknown. This study shows that up-regulation of miR-146a-5p can reduce CORT-induced apoptosis and oxidative stress response of HT-22 cells, while down-regulation of miR-146a-5p can promote CORT-induced apoptosis and oxidative stress response of HT-22 cells, indicating that miR-146a-5p is involved in the regulation of hippocampal nerve cell injury. This study also shows that hesperidin can promote the expression of miR-146a-5p in CORT-induced HT-22 cells, while down-regulation of miR-146a-5p reduce the inhibitory effect of hesperidin on CORT-induced apoptosis and oxidative stress of HT-22 cells, suggesting that hesperidin can inhibit CORT-induced apoptosis and oxidative stress

Table 4: Effects of miR-146a-5p upregulation on apoptosis and oxidative stress of CORT-induced HT-22 cells ($\bar{x}$±s, n=9)

Group	miR-146a-5p	Cleaved-caspase-3	MAD (μmol/g)	SOD (U/mg)	CAT (U/mg)	Apoptosis rate (%)
CORT+miR-NC	1.00±0.11	0.94±0.09	35.37±3.54	5.72±0.57	5.32±0.54	27.55±2.76
CORT+miR-146a-5p	2.67±0.27*	0.55±0.05*	23.76±2.38*	13.77±1.38*	8.89±0.89*	10.34±1.04*
F	17.184	11.364	8.165	16.175	10.288	17.505
p	0.000	0.000	0.000	0.000	0.000	0.000

Note: Compared with CORT+miR-NC group, *p<0.05.

Table 5: Effects of miR-146a-5p down-regulation on apoptosis and oxidative stress of CORT-induced HT-22 cells ($\bar{x}$±s, n=9)

Group	miR-146a-5p	Cleaved-caspase-3	MAD (μmol/g)	SOD (U/mg)	CAT (U/mg)	Apoptosis rate (%)
CORT+anti-miR-NC	1.00±0.12	0.97±0.10	35.07±3.51	5.68±0.59	5.48±0.55	26.96±2.70
CORT+anti- miR-146a-5p	0.42±0.04*	1.37±0.14*	44.27±4.43*	2.04±0.20*	1.98±0.20*	33.24±3.33*
t	13.756	6.975	4.883	17.529	17.942	4.395
p	0.000	0.000	0.000	0.000	0.000	0.000

Note: Compared with CORT+anti-miR-NC group, *p<0.05.

Table 6: Downregulation of miR-146a-5p reverses the effect of hesperidin on apoptosis and oxidative stress of CORT-induced HT-22 cells ($\bar{x}$±s, n=9)

Group	miR-146a-5p	Cleaved-caspase-3	MAD (μmol/g)	SOD (U/mg)	CAT (U/mg)	Apoptosis rate (%)
CORT+ hesperidin +anti-miR-NC	1.00±0.10	0.62±0.06	24.88±2.49	12.66±1.27	8.80±0.88	12.26±1.23
CORT+ hesperidin +anti-miR-146a-5p	0.48±0.05*	0.88±0.09*	32.24±3.23*	4.11±0.41*	4.68±0.47*	23.01±2.30*
t	13.953	7.211	5.414	19.220	12.389	12.365
p	0.000	0.000	0.000	0.000	0.000	0.000

Note: Compared with CORT+ hesperidin +anti-miR-NC group, *p<0.05.

of HT-22 cells by up-regulating the expression of miR-146a-5p, thus protecting cells from damage.

CONCLUSION

In conclusion, hesperidin can reduce CORT-induced apoptosis and oxidative stress response of rat HT-22 cells by upregulation of miR-146a-5p expression, and thus protect hippocampal nerve cells from being damaged.

ACKNOWLEDGEMENT

The research is supported by Qiqihar Science and Technology Bureau Project (No. sfgg-201760).

REFERENCES

Cao RG, Zhao YL, Zhou ZK, Zhou X (2018). Enhancement of the water solubility and antioxidant activity of hesperidin by chitooligosaccharide. *J. Sci. Food Agric.*, **98**(6): 2422-2427.

Freitas AE, Egea J, Buendía I, Navarro E, Rada P, Cuadrado A, Rodrigues ALS, Lopez M (2015). Agmatine induces Nrf2 and protects against corticosterone effects in hippocampal neuronal cell line. *Mol. Neurobiol.*, **51**(3): 1504-1519.

He XY, Chen JL, Xiang H, Gao Y, Tian JS, Qin XM, Du GH (2017). Comparative study of the corticosterone and glutamate induced PC12 cells depression model by 1H NMR metabolomics. *Acta Pharm. Sin.*, **52**(2): 245-252.

He YX, Lv BK, Huan YQ, Liu B, Li YT, Jia LZ, Qu CH, Wang DS, Yu H, Yuan HW (2018). Zhenbao pill protects against acute spinal cord injury via miR-146a-5p regulating the expression of GPR17. *Biosci. Rep.*, **38**(1):1-22

Iacona JR, Monteleone NJ, Lemenze AD, Cornett AL, Lutz CS (2019). Transcriptomic studies provide insights into the tumor suppressive role of miR-146a-5p in non-small cell lung cancer (NSCLC) cells. *RNA Biol.*, **16**(12): 1721-1732.

Kamboh AA, Zhu WY (2014). Individual and combined effects of genistein and hesperidin on immunity and intestinal morphometry in lipopolysacharide-challenged broiler chickens. *Poult. Sci.*, **93**(9): 2175-2183.

Lei N, Zhang XP, Wang C (2018). miR-381-3p alleviates cell damage in the hippocampus of depressed rats through BDNF. *Chin. J. Pathophysl.*, **34**(4): 699-704.

Long JP, Dong LF, Chen FF, Fan YF (2019). miR-146a-5p targets interleukin-1 receptor-associated kinase 1 to inhibit the growth, migration, and invasion of breast cancer cells. *Oncol. Lett.*, **17**(2): 1573-1580.

Menze ET, Tadros MG, Abdel-Tawab AM, Khalifa AE (2012). Potential neuroprotective effects of hesperidin on 3-nitropropionic acid-induced neurotoxicity in rats. *Neurotoxicol.*, **33**(5): 1265-1275.

Sun Y, Qu YC, Shen ZL (2017). Effect of corticosterone on zinc content in hippocampal HT-22 cell line and its mechanism. *Acta Nutrimenta Sin.*, **39**(2): 161-164.

Tejada S, Pinya S, Martorell M, Capó X, Tur JA, Pons A, Sureda A (2018). Potential anti-inflammatory effects of hesperidin from the genus citrus. *Curr. Med. Chem.*, **25**(37): 4929-4945.

Wang Y, Cai YJ, Huang HL, Chen XJ, Chen XY, Chen XT, Mai H, Li XH, Zhao JH, Yang JQ Fan WH Tang P, Chen YS, Li KS, Cui LL (2018). miR-486-3p influences the neurotoxicity of a-Synuclein by targeting the SIRT2 gene and the polymorphisms at target sites contributing to Parkinson's Disease. *Cell Physiol. Biochem.*, **51**(6): 2732-2745.

Wu Y, Xu J, Xu J, Cheng J, Jiao D, Zhou C, Dai Y, Chen Q (2017). Lower Serum Levels of miR-29c-3p and miR-19b-3p as Biomarkers for Alzheimer's Diseas. *Tohoku J. Exp. Med.*, **242**(2): 129-136.

Xiao HM, Liao JX, Jiang L (2013). Expression of miR-146a-5p and miR-23a-3p in serum of children with drug-resistant epilepsy. *Chin. J. Nerv. Ment. Dis.*, **39**(8):58-61.

Xu Y, Ye XL, Yan W (2019). Hesperidin alleviates rotenone-induced nerve cell damage. *Chin. J. Crit. Care Med.*, **12**(3): 182-186.

Yang H, Liu L, Du Q (2017). Effects of dizocyclopin on depression-like behavior and hippocampal neuron injury in diabetic rats with depression. *Chin. J. Behav. Med. Brain Sci.*, **26**(8): 684-688.

Yin CL, Han QJ, Xu DQ, Zheng BQ, Zhao XM, Zhang J (2019). SALL4-mediated upregulation of exosomal miR-146a-5p drives T-cell exhaustion by M2 tumor-associated macrophages in HCC. *Oncoimmunology*, **8**(7): 1601479

Zhang P, Jiang L, Liu XJ (2017). Damage to hippocampal neurons and synapses of rats with H2S antagonizes cortisol-induced depression. *Chin. J. Neuromedicine*, **16**(10): 1034-1040.

Zhang S, Zhang Y, Li B (2019). Protection effect of kaempferol on excessive autophagy and oxidative stress injury of model rats with H2S antagonizes cortisol-induced depression. *Chin. J. Immunol.*, **35**(2): 146-150.