Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

Clinical Pharmacy

The Effect of Isoquercitrin on Cell Apoptosis and Cycle for HepG2 Cells

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DOI: 10.36347/sajp.2022.v11i10.002 | **Received:** 09.10.2022 | **Accepted:** 24.11.2022 | **Published:** 29.11.2022

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Abstract **Original Research Article**

Purpose: To study the effect of isoquercitrin on cell apoptosis and cycle of HepG2 cells. Materials and Method: Different concentrations of isoquercitrin were used to act on HepG2 cells, and the effect of isoquercitrin on the proliferation of HepG2 cells was detected by CCK8. Cell morphology and growth were observed under inverted microscope. Flow cytometry was used to detect the apoptosis and cycle changes of HepG2 cells. Results: CCK8 found that isoquercitrin inhibited the growth of HepG2 cells, and it was correlated with the concentration and action time of isoquercitrin. Under the inverted microscope, it was observed that the number of cell survival gradually decreased with the increase of concentration or time of isoquercitrin acted on HepG2 cells. Flow cytometry showed that with the increase of isoquercitrin concentration, the number of cells blocked in S phase gradually decreased and the number of cells blocked in G2/M phase gradually increased. Conclusion: Isoquercitrin can induce apoptosis of HepG2 cells and interfere with S phase and G2/M phase in cell cycle.

Keywords: Isoquercitrin, HepG2, apoptosis, cycle, phase.

INTRODUCTION

Isoquercitrin is a flavonoid, and many studies show that it has many biological activities such as anti-inflammatory, anti-oxidation, anti-tumor and hypoglycemic [1, 2]. Nowadays, liver cancer is still a global challenge. As the number of patients with liver cancer continues to increase, medical resources and expenses also increase. Radiotherapy and chemotherapy are still the main treatment schemes for liver cancer [3]. It is found that isoquercitrin has a good anti-tumor effect, and its mechanism is related to its interaction with Wnt signal transduction pathway, mixed-lineage protein kinase-3, mitogen-activated protein kinase, apoptosis pathway and inflammatory protein signal [4]. In the previous research, we found that isoquercitrin can induce the apoptosis of liver cancer cells, and its main mechanism is to intervene the endoplasmic reticulum pathway in cells [5]. This experiment aims to study the effect of isoquercitrin on the apoptosis and cycle of liver cancer HepG2 cells.

MATERIALS AND METHODS

MATERIALS

HepG2 cells were purchased from the cell bank of Shanghai Institute of Life Sciences, Chinese Academy of Sciences. Isoquercitrin was purchased from Spring & autumn Company (purity ≥98%, no. 482-35-9). Serum was purchased from Ex-Cell Bio Company (no. 11E074). DMEM medium was purchased from Gibco Company (no. 8114351).

CCK8 assay

The logarithmic growth cells were inoculated into 96-well plates (2×10^3 cells per well) and cultured at 37℃ and 5% CO_2 for 24 hours. Different concentrations of isoquercitrin (0, 0.1, 0.2, 0.4, 0.8 mmol/L) were added into those 96-well plates, each group was provided with 6 multiple wells, and cultured for 24, 48, 72h. Two hours before the test, add 10 μL of CCK8 solution into each well, and then put the 96-well plate in a micro-plate reader at the wavelength of 450 nm to test the OD value of each well. The experimental results were analyzed according to the formula: the cell proliferation ratio= (OD drug group-OD control group)/ (OD blank group-OD control group).

Observation of cell morphological changes

Cells were collected by cell culture according to the above method, and cultured in 6-well plates (1×10^6 cells per well) for 24 hours. Different concentrations of isoquercitrin (0, 0.1, 0.2, 0.4 mmol/L)

were used to treat HepG2 cells for 24 hours and 48 hours, respectively, and then the growth and changes of HepG2 cells were observed under an inverted microscope.

Cell Apoptosis

HepG2 cells were cultured for 48 hours according to the above cell culture and grouping method. Then, each group of cells was made into cell suspension according to the cell subculture method, which was transferred to a centrifuge tube respectively, and centrifuged at 4℃ for 5 min at a speed of 1000 r/min. After centrifugation, the supernatant was sucked off, and 2 mL PBS was added for washing once. Centrifuge the cleaned cells again at the speed of 1000 r/min in a centrifuge at 4℃ for 5 min, and slowly blow the cells with a pipette to make a suspension. The isoquercitrin groups with different concentrations respectively absorb 50 μL of cell suspension, then add the same volume of Manix reagent for 20 min and detection.

Cell Cycle

HepG2 cells were cultured for 48 hours according to the above cell culture and grouping method. Then, each group of cells was made into cell suspension according to the cell subculture method, which was transferred to a centrifuge tube respectively, and centrifuged at 4℃ for 5 min at a speed of 1000 r,min. After centrifugation, the supernatant was sucked off, and 2 mL PBS was added for washing once. Centrifuge the cleaned cells in a centrifuge at 4℃ for 5 min, then discard the supernatant. Then, PBS was used for cleaning once according to the above method, PI dye solution was added, and dyed in the dark at room temperature for 30 min. According to the isoquercitrin concentration from low to high, it was put into a flow cytometer for detection.

Statistical Methods

The experimental data were analyzed by SPSS25 software. T test was used for comparison between the two groups, and variance analysis was used for comparison among multiple groups. An assessment of $p<0.05$ was considered statistically significant, and all experiments were repeated for 3 times.

RESULTS

The therapeutic effects of isoquercitrin on HepG2 cells viability

Isoquercitrin (0, 0.1, 0.2, 0.4, 0.8 mmol/L) can inhibit the growth of HepG2 cells, and with the increase of concentration and time, the inhibition gradually increases. When the action time is constant, the drug concentration is positively correlated with the proliferation ratio (Fig 1).

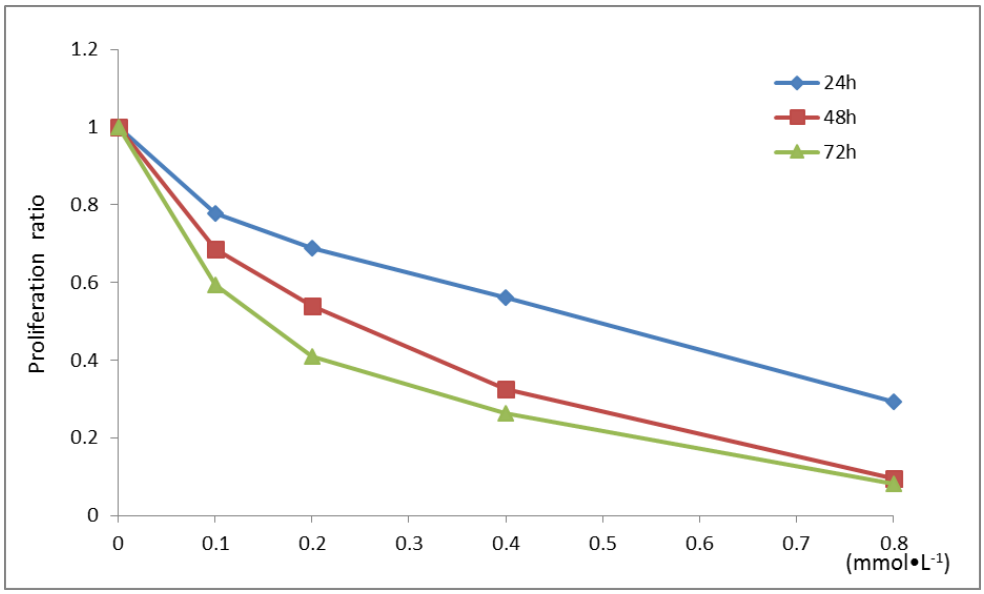

Figure 1: The growth effect of HepG2 cells treated with Isoquercitrin. Different concentrations of isoquercitrin (0, 0.1, 0.2, 0.4, 0.8 mmol/L) were used to treat HepG2 for 24, 48, and 72 h

Cell growth and morphological changes

Isoquercitrin (0, 0.1, 0.2, 0.4 mmol/L) can inhibit the growth and change in the morphology of HepG2 cells. As shown in Figure 2, different concentrations of isoquercitrin act on HepG2 cells for different time under an inverted microscope. Compared with the control group, the number of suspended cells gradually increases with the increase of the concentration or the extension of the acting time of isoquercitrin.

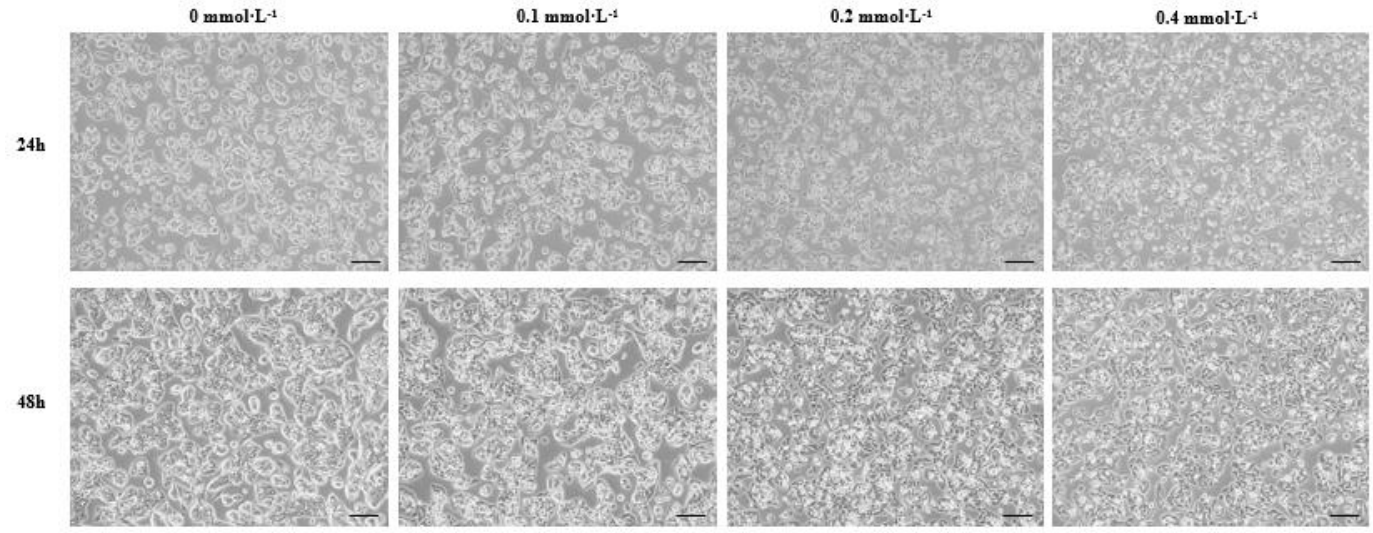

Figure 2: Isoquercitrin inhibits HepG2 cells proliferation was observed by inverted microscopy. HepG2 cells were treated with different concentrations of isoquercitrin (0, 0.1, 0.2, 0.4 mmol/L) for 24, and 48 h

Changes of apoptosis in HepG2 cells

Isoquercitrin can change the percentage of apoptosis. Compared with the control group, the percentage of HepG2 apoptotic cells increased when the isoquercitrin concentration was 0.1 mmol/L. However, with the increase of isoquercitrin concentration, the percentage of apoptosis detected decreased gradually. When isoquercitrin concentration was 0.4 mmol/L, compared with the blank control group, the percentage of apoptosis detected by HepG2 decreased, and the difference was statistically significant (Fig 3).

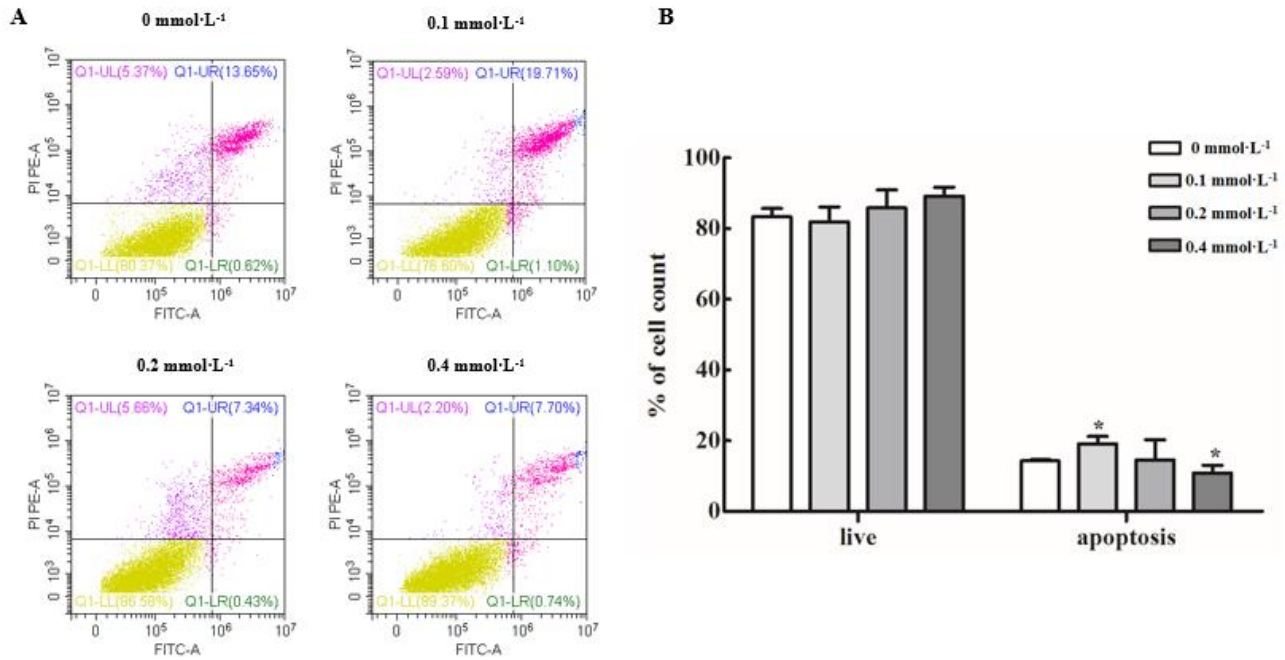

Figure 3: The effect of HepG2 cells was treated with isoquercitrin on Cell apoptosis. A: The apoptosis of HepG2 cells treated with isoquercitrin (0, 0.1, 0.2, 0.4 mmol/L) for 48h was detected by flow cytometry; B: The percentage of apoptosis of HepG2 cells treated with isoquercitrin for 48 hours. *P<0.05 vs control

Changes of cycle in HepG2 cells

Isoquercitrin can cause the change of cell cycle. Compared with the control group without isoquercitrin, the percentage of cells blocked in G2/M phase of HepG2 cells gradually increased, and the percentage of cells blocked in S phase gradually decreased (Fig 4).

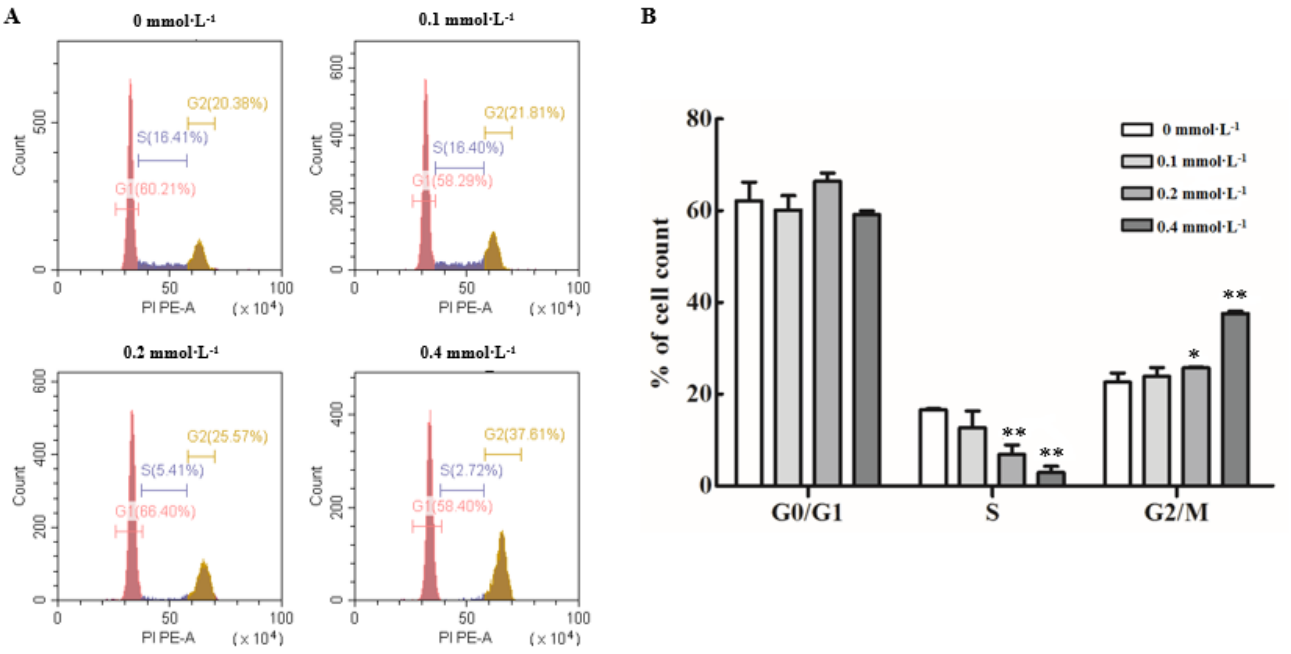

Figure 4: The effect of HepG2 cells was treated with isoquercitrin on Cell cycle distribution. A: The cell cycle of HepG2 cells treated with isoquercitrin (0, 0.1, 0.2, 0.4 mmol/L) for 48h was detected by flow cytometry; B: Isoquercitrin blocks HepG2 cell cycle in the G0/G1、S and G2/M phases. *P<0.05 vs control, **P<0.01 vs control

DISCUSSION

Recently, the increase of cancer incidence, the price increase of several chemotherapeutic drugs with unexpected side effects, and the multidrug resistance to cancer cells have brought great challenges to cancer treatment [3]. Therefore, there is an urgent need to develop safe chemotherapy, including plant-based natural drugs as effective anticancer agents, because they are easily available and cause minimal side effects [6].

Isoquercitrin can promote the apoptosis of human hepatoma cells and inhibit the proliferation of human hepatoma cells by blocking the cell cycle [7]. Studies have shown that isoquercitrin can activate caspase-3, caspase-8 and caspase-9, inhibit the expression level of ERK and p38MAPK phosphorylated proteins, promote JNK phosphorylation, and reduce the expression level of protein kinase C in cancer cells [8]. Other studies have shown that isoquercitrin can reduce the growth of liver cancer cells by inhibiting the expression of Wnt /β- catenin and regulating the control of cell cycle [9]. This is consistent with the possible mechanism of isoquercitrin induce HepG2 cells.

Cell cycle refers to the whole process of a cell from the completion of one division to the end of the next division, which is divided into two stages: interphase and mitosis [10]. There are two most important stages in the cell cycle: G1 to S and G2 to M. Both stages are in a period of complex and active molecular level changes, which are easily influenced by environmental conditions [11]. It is significant for biological growth and controlling tumor growth if it can be controlled artificially [12].

In this experiment, we found that isoquercitrin can inhibit the growth of HepG2 cells. With the prolongation of the action time or the increase of the concentration of isoquercitrin, the number of HepG2 cells gradually decreased and the number of cells with morphological changes gradually increased. Flow cytometry showed that the percentage of apoptosis of HepG2 cells increased gradually when isoquercitrin reached a certain concentration, but the percentage of apoptosis of HepG2 cells decreased gradually in the high-concentration group, which was mainly related to excessive cell damage. The detection of HepG2 cell cycle showed that with the increase of drug concentration, the percentage of cells whose apoptosis was blocked in the early stage had no obvious change, the percentage of cells blocked in the middle stage gradually decreased, and the percentage of cells blocked in the later stage gradually increased.

CONCLUSION

Through these studies, the research group confirmed that isoquercitrin has anti-cancer activity on HepG2 cells and interfere with S phase and G2/M phase in cell cycle. In addition, the work on overexpression of various inhibitors or genes with sustainable mechanisms is currently underway. Further in vivo studies in animal models are needed to clarify the mechanism of isoquercitrin.

ACKNOWLEDGMENTS

This work were supported by grants from the Project of improving the Scientific Research ability of the Young and Middle-aged staff of Guilin Medical College (NO. 2018glmcy107), the National Natural Science Foundation of China (NO.81260661,81860658, 82160701), the Guangxi natural science foundation (No.2018gxnsfa281168), the Guilin scientific research and technology development plan project (NO.20140120-1-9, 20170109-47, 20210227-5), the Medical Science Research Fund of Beijing Medical Health Public Welfare Foundation (No.YWJJJJJJJJJJ-B184007, YWJJJJJJJJ-B20197DS, YWJJJJJJJJJ-B20234DS), and the Guilin Medical University education teaching research and reform (No. JGZ202116).

REFERENCES

1. Chen, Y., Zhan, Y., Li, C., Xu. F., & Zhao, J. (2018). Research progress on biological activity of isoquercitrin. *Modern Chinese Medicine*, *20*(12), 5.
2. Orfali, G., Duarte, A. C., Bonadio, V., Martinez, N. P., & Priolli, D. G. (2016). Review of anticancer mechanisms of isoquercitin. *World Journal of Clinical Oncology*, *7*(2), 11.
3. Hepatocellular carcinoma. (2021). *Nature reviews. Disease primers*, *7*(1), 7.
4. Xie, J., Wang, Y., Jiang, W. W., Luo, X. F., & Sheng, J. (2018). Moringa oleifera Leaf Petroleum Ether Extract Inhibits Lipogenesis by Activating the AMPK Signaling Pathway. *Frontiers in pharmacology*, *9*, 1447.
5. Jiang, G., Liu, T., Wu, T., Huang, J., & Huang, G. (2019). Isoquercitrin inhibits the progression of hepg2 cells via the endoplasmic reticulum stress. *Latin American journal of pharmacy*, *38*(1), 182-190.
6. Shukla, S., & Mehta, A. (2015). Anticancer potential of medicinal plants and their phytochemicals: a review. *Brazilian Journal of Botany*, *38*(2), 199-210.
7. Hintze, O., Gerber, B., Reimer, T., & Richter, D. U. (2020). Effect of Turmeric and its ingredients Curcumin, Caffeic Acid, Bisdemethoxycurcumin, Isoquercitrin and Quercetin on malignant hormone receptor-positive Breast Cancer Cells (MCF7). *Geburtshilfe und Frauenheilkunde*, (10), 80.
8. Li, L. I., Zhang, X. H., Liu, G. R., Liu, C., & Dong, Y. M. (2016). Isoquercitrin suppresses the expression of histamine and pro-inflammatory cytokines by inhibiting the activation of MAP Kinases and NF-κB in human KU812 cells. *Chinese Journal of Natural Medicines*, 14(6), 0407- 0412.
9. Amado, N. G., Predes, D., Fonseca, B. F., Cerqueira, D. M., Reis, A. H., Dudenhoeffer, A. C., ... & Abreu, J. G. (2014). Isoquercitrin suppresses colon cancer cell growth in vitro by targeting the Wnt/β-catenin signaling pathway. *Journal of Biological Chemistry*, *289*(51), 35456-35467.
10. Morenolayseca, P., & Streuli, C. H. (2013). Signalling pathways linking integrins with cell cycle progression. *Matrix biology: journal of the International Society for Matrix Biology*, *34*(4), 144-153.
11. Engstrm, Y. (2021). Cell cycle regulators control stemness and differentiation. *BioEssays: news and reviews in molecular, cellular and developmental biology*, (6).
12. Ding, F. F., Zhang, X. J., & Deng, Y. R. (2014). Inhibition of periplocin on human hepatoma carcinoma and breast carcinoma cells in vitro. *Drug Evaluation Research*.