

Dose dependent cytotoxic activity of patulin on neuroblastoma, colon and breast cancer cell line

 Nese Basak Turkmen¹,  Hande Yuce¹,  Dilan Askin Ozek²,  Sumeyye Aslan¹,  Seyma Yasar³,  Songul Unuvar¹

¹Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Inonu University, Malatya, Turkey

²Department of Pharmacy Services, Kovancilar Vocational School, Firat University, Elazig, Turkey

³Department of Biostatistics, Faculty of Medicine, Inonu University, Malatya, Turkey

Copyright@Author(s) - Available online at www.annalsmedres.org

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.



Abstract

Aim: Patulin, a mycotoxin, is an organic compound classified as a polypeptide. Patulin, which is generally detected in moldy fruits and their derivatives, has been suggested to have anticancer activity. Some studies have shown that it induces apoptosis in the cell. This study aims to investigate the anticancer activity of patulin in SH-SY5Y (human neuroblastoma cell line), HCT116 (human colon cancer cell line), and MCF-7 (human breast cancer cell line) cell lines.

Materials and Methods: SH-SY5Y, HCT116, MCF-7, and L929 (healthy fibroblast) cell lines were used for cytotoxicity experiments. Cells were added in 96-well plates at 5x10³ cells per well. Serial dilutions of patulin at a dose of 1, 2.5, 5, 10, 25, 50, and 100 µM were added to the waiting cells in 24 hours incubation. All cell lines were exposed to patulin for 24 and 48 hours. The cytotoxic activity of patulin in cancer and healthy cell lines was determined in vitro by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium) cell viability test. The results of the toxicity tests were measured spectrophotometrically (450 nm) in ELISA at intervals of 24 hours for 2 days.

Results: Patulin caused cytotoxic activity in all cell lines at a concentration of 100 µM. Patulin showed cytotoxic activity at low doses only in the SH-SY5Y cell line. At doses of 25 and 50 µM, HCT116 caused more than 50% death in the cell line, while higher concentrations induced cell death in the MCF-7 cell line.

Conclusion: Patulin showed anticancer activity at high concentrations in colon and breast cancer cell lines, and both low and high concentrations in the SH-SY5Y cell line. Patulin may be a new candidate molecule in the treatment of neuroblastoma, colon, and breast cancers, depending on the dose.

Keywords: HCT116; MCF-7; mycotoxin; patulin; SH-SY5Y cytotoxicity

INTRODUCTION

Patulin (4-Hydroxy-4H-furo 3,2-C-pyran-2 (6H) -one) is produced by various types of fungi such as *Penicillium*, *Aspergillus*, and *Byssochlamys*. Patulin is the secondary metabolite of *Penicillium* and *Aspergillus*, including *A. clavatus*, *P. patulum*, *P. aspergillus*, *P. byssochlamys*, and *P. expansum* (1). The main source of human exposure to patulin is contaminated food products such as fruits, juices, vegetables, cereals, and cheese (2). The low amount of patulin in apples used in fruit juice or compost production increases the quality level of the beverage (3). Patulin was found to be associated with immunotoxicity, genotoxicity, embryotoxicity, and teratogenicity (4-7), however, studies on the anticancer activity of patulin also available (8-10). The mechanism of action of the anticancer activity of patulin, the types of cancer it is effective on and the doses are not fully known. In some studies, it has been suggested that it induces cellular apoptosis and cytotoxicity by

mechanisms related to reactive oxygen radicals (ROS) (11). Owing to its electrophilic feature, patulin can bind to sulfhydryl groups of RNA, glutathione, and proteins by covalent bonding, resulting in glutathione consumption and inhibition of RNA and protein synthesis (11). Thus, oxidative damage and mitochondrial dysfunction can cause cytotoxicity by activating mitochondrial apoptotic signaling pathways (12-14). There are few reports of the effects of patulin on human cell lines, particularly cancer cells.

Current drugs used in cancer treatment have serious side effects such as neutropenia, immunosuppression, infertility, peripheral neuropathy, heart, kidney and liver damage. For this purpose, researchers have focused on investigating the effectiveness of toxins obtained from bacteria or fungi found in nature in cancer treatment (9,15,16).

Received: 26.01.2021 **Accepted:** 17.03.2021 **Available online:** 21.09.2021

Corresponding Author: Nese Basak Turkmen, Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Inonu University, Malatya, Turkey **E-mail:** nesebasak86@gmail.com

This study aims to determine the cytotoxic effect of patulin on human neuroblastoma, colon, and breast cancer cell lines in vitro. Determining the potential anticancer activity of patulin will contribute to new studies in this area.

MATERIALS and METHODS

Cell Culture

Patulin was obtained from Toronto Research Chemicals company (Cas No: 149-29-1). In our study, four different cancer cells were used with the anticancer activity of patulin: healthy fibroblast cell line (L929), human colon cancer cell line (HCT-116), breast cancer cell line (MCF-7), and neuroblastoma cell line (SH-SY5Y). Human cancer cell lines were purchased from the American Type Culture Collection (ATCC). Cell lines were inoculated in T-75 flasks with Dulbecco's Modified Eagles Medium (DMEM) containing 1% penicillin-streptomycin and 10% fetal calf serum. The DMEMs of the cells left in the incubator (5% CO₂ and 37°C; NUVE) were changed every three days. When the cells reached 90% density, the cells were removed from the T-75 flasks using trypsin enzyme (TrypLE™ Express, Invitrogen, Carlsbad, CA). Neutralization was done with DMEM (1: 1). Cells precipitated by centrifugation (800 rpm, 5 min) were homogeneously dispersed with DMEM. The number of viable cells was determined by hemocytometer and Trypan blue (0.4%) staining. Cells were added to 96 wells as 5x10³ in each well. Cells were kept at 37 °C and 5% CO₂ in the incubator for 24 hours.

Cell Viability Assay

Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl) -5-(3-carboxymethoxyphenyl) -2-(4-sulphophenyl) -2H-tetrazolium (MTS) method. Medium was removed from the cells. Malignant cells and healthy fibroblast cells were incubated with serial dilutions of patulin (1, 2.5, 5, 10, 25, 50, and 100 µM) for 48 hours. The color change at the end of the specified periods was measured with an ELISA microplate reader (DRG Diagnostics GmbH, Germany) at 450 nm wavelength. In this study, our cell proliferation values were obtained by proportioning the absorbance values obtained from negative controls in the ELISA microplate reader to the absorbance values of cancer cell lines in%. The absorption value obtained from controls (cells not treated with patulin) was accepted as 100% cell viability. The MTS trials were repeated four times for each concentration at 24 and 48 hours (n: 4) (17).

Statistical Analysis

Statistical analysis was done with SPSS 22.0 package program. The homogeneity of variances was checked with the "Levene" test. Normality assumption was analyzed using the "Shapiro-Wilk" test. Differences between groups were determined by the Kruskal Wallis test. IC₅₀ values were calculated using the GraphPad Prism 8 program. The significance limit was accepted as p < 0.001.

RESULTS

The cytotoxic effect of patulin on the SH-SY5Y, HCT-116, and MCF-7 cell line determined by MTS after 24 and

48 hours incubation at concentrations of 1, 2.5, 5, 10, 25, 50, 100 µM was showed in figures. Patulin showed cytotoxic activity in L929, HCT-116, MCF-7, and SH-SY5Y cell lines at a concentration of 100 µM.

After the SH-SY5Y was incubated with different concentrations of patulin for 48 hours, the % changes in the viability of the cells were determined and the results are shown in Figure 1. Patulin showed cytotoxic activity in L-929 and SH-SY5Y cell lines at low concentrations. The strongest cytotoxic effect was seen at concentrations of 5 µM and 10 µM. Low concentration of patulin (1 µM and 2.5 µM) showed less than 50% cell viability in SH-SY5Y cells compared to healthy fibroblast cells (65%). At concentrations of 10 µM and 100 µM, it showed more cytotoxic effects on cancer cells compared to healthy cells. However, this rate is less than 5% in both concentrations. The IC₅₀ value of L-929 was calculated to be 12.48 µM and the IC₅₀ value of SH-SY5Y was calculated as 2.5 µM. The microscopic views of SHSY-5Y cells 48 hours after the administration of patulin are shown in Figure 2.

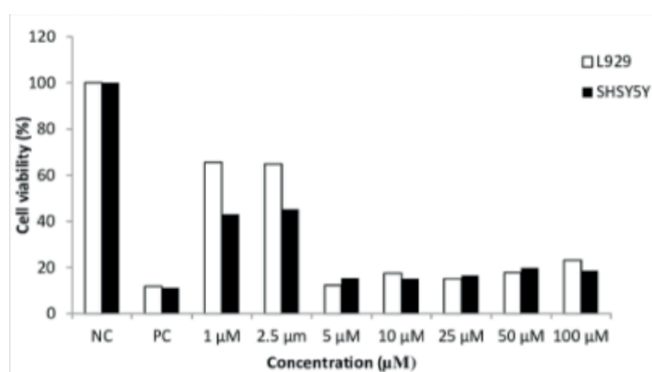


Figure 1. Cytotoxic effect of patulin on the SH-SY5Y and L-929 cell line

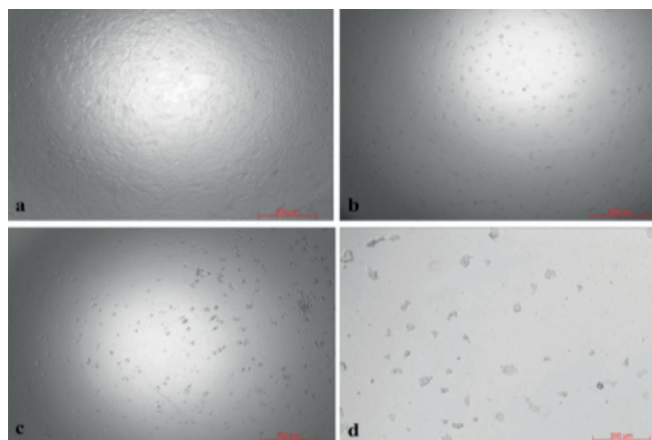


Figure 2. Microscopic views of SHSY-5Y cells 48 hours after the administration of patulin

After HCT-116 was incubated with different concentrations of patulin for 48 hours, the % changes in the cell viability were determined and the results are shown in Figure 3. While patulin caused a decrease in viability of healthy cells at all doses, it did not show the same effect on HCT-116

cells. It showed cytotoxic activity on malignant cells at high concentrations (50 and 100 μM). HCT-116 cells were found to have lower viability (16%) than healthy fibroblasts (18%) at 50 μM concentration. It showed significant activity at a concentration of 100 μM . The survival rate of healthy fibroblasts is 36% while the survival rate of HCT-116 cells is 13%. The IC_{50} value was calculated as 23.9 μM (Figure 3).

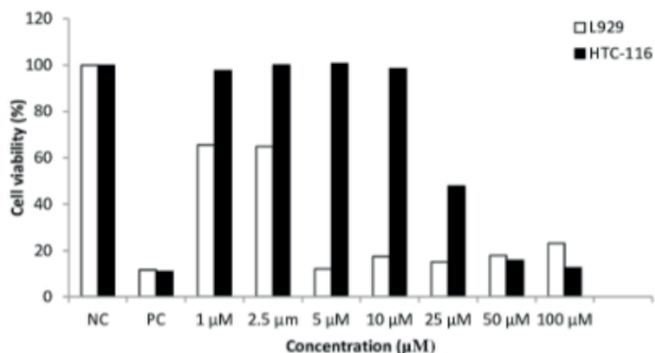


Figure 3. Cytotoxic effect of patulin on the HCT-116 and L-929 cell line

While the viability of healthy cells decreased more than MCF-7 cells at all concentrations except patulin concentration of 100 μM ; At 100 μM concentration, the viability of healthy cells (36%) was higher than that of MCF-7 cells (12%) (Figure 4).

The survival of healthy cells at a concentration of 100 μM can be explained by the inadequate diffusion of patulin into the fibroblast. The IC_{50} value for this cell line was calculated to be 25 μM .

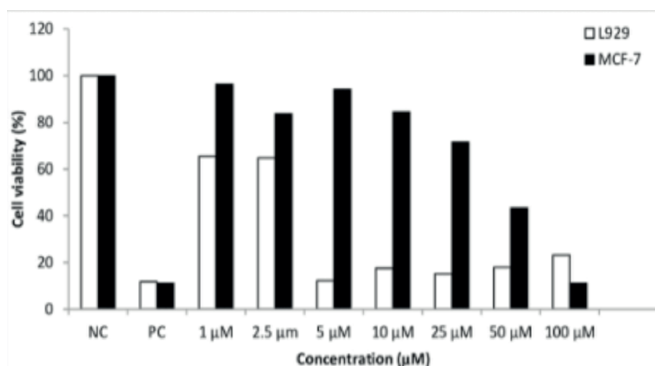


Figure 4. Cytotoxic effect of patulin on the MCF-7 and L-929 cell line

DISCUSSION

According to the International Agency for Research on Cancer (IARC) data, patulin is in Group 3, which lists compounds that are not classified as carcinogens in humans (18). Since it has no carcinogenic effect on healthy cells, the cytotoxic effect of patulin in different cancer cell lines has been investigated. Patulin has been suggested to show anticancer activity in cervical and colorectal cancer cell lines (HeLa, SW-48 and MRC-5), human leukemia cell line (HL-60), hepatocellular carcinoma cell line (HepG2) (8-10).

Abastabar et al. (16) showed that patulin inhibits cell proliferation in colorectal and cervical cancer cell lines. Another study suggested that patulin stopped cell division at the G2-M stage in the HCT-116 cell line at 0-10 μM concentrations (9). According to Wu et al., (19) the antitumoral effect of patulin on the promyelocytic (HL-60) cell line occurs via caspase proteins, an apoptotic pathway.

Patulin damages macromolecules such as protein, enzyme, DNA, and RNA by stimulating the production of ROS in the cell. Therefore, it causes genotoxicity, embryotoxicity, cytotoxicity, neurotoxicity, immunotoxicity, carcinogenicity, and teratogenicity (4-8). Increased ROS reduces the activity of the antioxidant defense system inside the cell, thus activating apoptotic pathways. Patulin impairs cell membrane permeability, causing abnormalities in mitochondrial membrane potential and damage to mitochondrial respiratory chain complexes, leading to cell apoptosis (14, 20).

Patulin reacts with glutathione and its metabolite N-acetyl-L-cysteine in SH-SY5Y cells, causing toxicity and ROS production. This may lead to altered patulin stability or better penetration into the mammalian cell. In addition, patulin can significantly reduce the viability of neuronal cells by inhibiting NF- κ B activity by 90% even at concentrations as low as 0.5 micromolar (21).

Kwon et al., In a study using HCT-116 colon cancer cell line; It has been suggested that patulin may be a candidate for the treatment of colorectal cancers (22). However, it was found that patulin administration in the HCT-116 cell line affects the mitochondrial membrane potential and the release of cytochrome-C into the cytoplasm, thus inducing caspase 3 activation, causing apoptosis of human colon carcinoma cells (23). The differences in the results obtained from both studies were related to the applied patulin dose.

CONCLUSION

In conclusion, in this study, the cytotoxic activity of Patulin at different concentrations in colon cancer, breast cancer, and neuroblastoma cell lines was compared with the control group (L-929 cell line) and was determined by the MTS experiment. Although the pathway through which patulin exerts its antitumoral activity has not been fully elucidated, we think that patulin can be used as an antitumoral agent as a result of literature reviews and our study. No literature data is examining the relationship between patulin and breast cancer cell line, our study presents the first data on this subject. Therefore, we consider that patulin may be a candidate molecule for colon and breast cancer cell lines. The fact that the cancer cell lines used in the study are human-specific increases the importance of the results we obtained from our study. More studies are needed both in vitro and in vivo to better understand the anticancer effect mechanisms of patulin.

***3rd International Hippocrates Medicine and Health Sciences Congress (6-7 March 2020) Presented as an oral presentation in Ankara.

Competing Interests: The authors declare that they have no competing interest.

Financial Disclosure: We acknowledge the support of IUBAP (Scientific Research Fund of Inonu University) under Grant TSG-2020-2087.

Ethical Approval: The Ethics Committee Approval Document Not Required According to the Clause 1 of the First Part of the "Regulation on Clinical Studies" dated April 13, 2013 and numbered 28617, (This Regulation covers clinical trials, clinical research sites and real or legal persons who will carry out these researches, including bioavailability and bioequivalence studies, including medicines, medicinal and biological products and herbal products to be carried out on humans even if licenses or permits have been obtained.) the Ethics Committee has not taken a decision since the study is not a clinical research but only a laboratory study.

REFERENCES

- Davis ND, Diener UL. Mycotoxins, Food and Beverage Mycology, L.R. Beuchat, AVI Publishing Company, INC., Westport, Connecticut 1978; 397-44.
- Liu BH, Wu TS, Yu FY, et al. Mycotoxin patulin activates the p38 kinase and JNK signaling pathways in human embryonic kidney cells. *Toxicol Sci* 2006;89:423-30.
- Zhong L, Carere J, Lu Z, et al. Patulin in apples and apple-based food products: the burdens and the mitigation strategies. *Toxins* 2018;10:475.
- Puel O, Galtier P, Oswald IP. Biosynthesis and toxicological effects of patulin. *Toxins* 2010;2:613-31.
- Roll R, Matthiaschk G, Korte A. Embryotoxicity and mutagenicity of mycotoxins. *J. Environ. Pathol. Toxicol. Oncol* 1990;10:1-7.
- Sakai M, Abe K, Okumura H, et al. Genotoxicity of fungi evaluated by SOS microplate assay. *Nat. Toxins* 1992;1:27-34.
- Ciegler A, Beckwith AC, Jackson LK. Teratogenicity of patulin and patulin adducts formed with cysteine. *Appl. Environ. Microbiol* 1976;31:664-67.
- Liu BH, Yu FY, Wu TS, et al. Evaluation of genotoxic risk and oxidative DNA damage in mammalian cells exposed to mycotoxins, patulin and citrinin. *Toxicol Appl Pharmacol* 2003;191:255-63.
- Kwon O, Soung NK, Thimmegowda NR, et al. Patulin induces colorectal cancer cells apoptosis through EGR-1 dependent ATF3 up-regulation. *Cell Signal* 2012;24:943-50.
- Fernández-Blanco C, Elmo L, Waldner T, et al. Cytotoxic effects induced by patulin, deoxynivalenol and toxin T2 individually and in combination in hepatic cells (HepG2). *Food Chem Toxicol* 2018;120:12-23.
- Pal S, Singh N, Ansari KM. Toxicological effects of patulin mycotoxin on the mammalian system: an overview. *Toxicol Res* 2017;6:764-71.
- Fliege R, Metzler M. Electrophilic properties of patulin N-acetylcysteine and glutathione adducts. *Chem Res Toxicol* 2000;13:373-81.
- Ayed Boussema I, Abassi H, Bouaziz C, et al. Antioxidative and antigenotoxic effect of vitamin E against patulin cytotoxicity and genotoxicity in HepG2 cells. *Environ Toxicol* 2013;28:299-306.
- Zhang B, Peng X, Li G, et al. Oxidative stress is involved in patulin induced apoptosis in HEK293 cells. *Toxicon* 2015;94:1-7.
- Wang X, Jin C, Zhong Y, et al. Glutathione reduction of patulin-evoked cytotoxicity in HEK293 cells by the prevention of oxidative damage and the mitochondrial apoptotic pathway. *J Agric Food Chem* 2018;66:7775-85.
- Abastabar M, Akbari A, Akhtari J, et al. In vitro antitumor activity of patulin on cervical and colorectal cancer cell lines. *Curr Med Mycol* 2017;3:25-9.
- Demirci S, Dogan A, Turkmen NB, et al. Schiff base-Poloxamer P85 combination demonstrates chemotherapeutic effect on prostate cancer cells in vitro. *Biomed Pharmacother* 2017;86:492-501.
- Sung B, Prasad S, Yadv VR, et al. Cancer cell signaling pathways targeted by spice-derived nutraceuticals. *Nutr Cancer* 2012;64:173-97.
- Wu TS, Liao YC, Yu FY, et al. Mechanism of patulin-induced apoptosis in human leukemia cells (HL-60). *Toxicol Lett* 2008;183:105-11.
- Zhong Y, Jin C, Gan J, et al. Apigenin attenuates patulin-induced apoptosis in HEK293 cells by modulating ROS-mediated mitochondrial dysfunction and caspase signal pathway. *Toxicon* 2017;137:106-13.
- Brand B, Stoye NM, dos Santos Guilherme M, et al. Identification of patulin from penicillium coprobium as a toxin for enteric neurons. *Molecules* 2019;24:2776.
- Kwon O, Soung NK, Thimmegowda NR, et al. Patulin induces colorectal cancer cells apoptosis through EGR-1 dependent ATF3 up-regulation. *Cell Signal* 2012;24:943-50.
- GuerraMoreno A, Hanna J. Induction of proteotoxic stress by the mycotoxin patulin. *Toxicol Lett* 2017;276:85-91.