DOI: 10.24938/kutfd.1693317 Kırıkkale Üniversitesi Tıp Fakültesi Dergisi 2025;27(2):192-196
Original Article *Özgün Araştırma*

RESERPINE ENHANCES THE GENOTOXIC EFFECTS OF DOXORUBICIN IN HEPATOCELLULAR CARCINOMA CELLS

Reserpin Hepatosellüler Karsinom Hücrelerinde Doksorubisinin Genotoksik Etkilerini Artırır

Sümeyra ÖZDOĞRU[1] Sedef Nur ŞENGÜL[1] Enes SAYIN[1] Beyza Nur BIÇER[1]
Safa Burak ARSLAN[1] Zülal BUCAK[1] Erkan YURTCU[2]

[1]*Kırıkkale University, Faculty of Medicine, Student, KIRIKKALE, TÜRKİYE*
[2]*Department Of Medical Biology, Faculty of Medicine, TOBB ETÜ, ANKARA, TÜRKİYE*

ABSTRACT

Objective: Hepatocellular carcinoma is the most frequently diagnosed primary liver malignancy. Despite advances in treatment, challenges such as drug resistance limit the effectiveness of chemotherapy, including doxorubicin. Studies have shown that reserpine, a naturally occurring indole alkaloid, has anticancer properties in various malignancies. The aim of this study was to investigate whether reserpine increases the effectiveness of doxorubicin in hepatocellular carcinoma cells under *in vitro* conditions.

Material and Methods: Human hepatocellular carcinoma (HepG2) cells were cultured. The IC_{50} value of reserpine was determined by the MTT method. Doxorubicin and reserpine were applied to HepG2 cells, both separately and in combination. DNA damage was evaluated by the alkaline comet assay.

Results: Doxorubicin and reserpine significantly increased DNA damage in HepG2 cells when applied alone ($p<0.05$). When administered together with doxorubicin, reserpine significantly increased the genotoxic effect of doxorubicin on HepG2 cells ($p<0.05$).

Conclusion: Reserpine increased the genotoxic effects of doxorubicin on HepG2 cells *in vitro*. This suggests that reserpine can be used as an adjuvant therapy to increase the therapeutic efficacy of doxorubicin in the treatment of hepatocellular carcinoma. Further studies are needed to better understand the basic mechanisms and possible clinical benefits of this combination.

Keywords: *Hepatocellular carcinoma, reserpine, doxorubicin, genotoxicity*

ÖZ

Amaç: Primer karaciğer maligniteleri arasında en sık tanı konulanı hepatosellüler karsinomdur. Tedavideki ilerlemelere rağmen, ilaç direnci gibi zorluklar doksorubisin dahil olmak üzere kemoterapilerin etkinliğini sınırlandırmaktadır. Yapılan çalışmalarda doğal olarak oluşan bir indol alkaloidi olan reserpinin çeşitli malignitelerde antikanser özellikler göstermiştir. Bu çalışmada amacımız, *in vitro* koşullarda reserpinin hepatosellüler karsinom hücrelerinde doksorubisinin etkinliğini artırıp artırmadığı araştırılmasıdır.

Gereç ve Yöntemler: İnsan hepatosellüler kanser hücreleri (HepG2) kültüre edildi. Rezerpinin IC_{50} değeri MTT testi ile belirlendi. Doksorubisin ve reserpin HepG2 hücrelerine hem ayrı ayrı hem de birlikte uygulandı. DNA hasarı ise alkali comet testi ile değerlendirildi.

Bulgular: Doksorubisin ve rezerpin tek başına uygulandığında HepG2 hücrelerinde DNA hasarını anlamlı düzeyde artırdı ($p<0,05$). Doksorubisin ile birlikte uygulandığında, rezerpin doksorubisinin HepG2 hücreleri üzerindeki genotoksik etkisini anlamlı şekilde artırdı ($p<0,05$).

Sonuç: Reserpin, *in vitro* ortamda HepG2 hücrelerinde doksorubisinin genotoksik etkilerini artırmıştır. Bu durum, hepatosellüler karsinoma tedavisinde doksorubisinin terapötik etkinliğini artırmak amacıyla reserpinin adjuvan bir tedavi olarak kullanılabileceğini düşündürmektedir. Bu kombinasyonun temel mekanizmalarının ve olası klinik faydalarının daha iyi anlaşılabilmesi için ileri araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: *Hepatosellüler karsinom, rezerpin, doksorubisin, genotoksisite*

Correspondence / Yazışma Adresi: **Dr. Erkan YURTCU**
Department Of Medical Biology, Faculty of Medicine, Kırıkkale University, KIRIKKALE, TÜRKİYE
Phone / Tel: +905335735434 **E-mail** / E-posta: erkanyurtcu@gmail.com
Received / Geliş Tarihi: 09.05.2025 **Accepted** / Kabul Tarihi: 18.05.2025

KÜ Tıp Fak Derg 2025;27(2):192-196
Doi: 10.24938/kutfd.1693317

INTRODUCTION

Reserpine, a naturally occurring compound extracted from *Rauwolfia serpentina* or *Rauwolfia vomitoria*, species endemic to the Indian subcontinent and East Asia, is a dopamine depletor known to inhibit several neurotransmitters.[1] As an indole alkaloid, reserpine functions not only as a drug efflux pump inhibitor but also exhibits potent antioxidant and antiangiogenic properties.[2] Clinically, reserpine has been used to manage hypertension, schizophrenia, insomnia, and other psychiatric conditions.[1] When administered alongside diuretics, it effectively reduces arterial pressure and significantly decreases hypertension-related morbidity and mortality.[3] Initial studies beginning in the mid-1950s reported that reserpine may have *in vivo* anticancer effects independent of its cardiovascular effects. Experimental studies on mice with advanced leukemia demonstrated that reserpine significantly extended survival. Other studies also reported *in vivo* anticancer activity of reserpine in various murine sarcomas.[3]

Among primary malignant liver tumors, hepatocellular carcinoma (HCC) is the most frequently diagnosed tumor.[4] It is the sixth most diagnosed cancer worldwide and ranks third in cancer-related mortality. In 2022, approximately 750,000 individuals died due to liver cancer.[5] The stepwise progression of HCC involves chronic liver damage, inflammation, hepatocellular degeneration, necrosis, and dysplasia. Eventually, HCC develops from low- and high-grade dysplastic nodules.[6,7] Molecular profiling analyses have defined two main subtypes of HCC. One is the proliferation class, associated with poor outcomes, genomic instability, and HBV infection, while the other is the non-proliferation class, associated with better outcomes and characterized by alcohol or HCV etiology.[8,9] Despite advancements in understanding the molecular basis of HCC, many mutations occur in pathways that cannot be targeted with drugs, complicating targeted therapy efforts.[10,11]

HCC may be treated with localised therapies if identified promptly. Nevertheless, HCC is frequently identified at advanced stages when the tumor is unresectable, rendering these treatments ineffective.[12] A variety of therapeutic strategies are available for hepatocellular carcinoma, ranging from liver transplantation and surgical resection to percutaneous ablation, radiotherapy, and systemic or trans-arterial therapies.[13]

Doxorubicin, a chemotherapeutic agent used in trans-arterial therapy for HCC, is an anthracycline antibiotic widely employed as an antitumor therapeutic agent and can induce immunogenic cell death.[14] Upon entering the cell, doxorubicin penetrates the nucleus and interacts with DNA, triggering a cascade of signaling pathways that result in programmed responses culminating in apoptosis. Doxorubicin exerts multiple antitumor effects and is a strong inhibitor of topoisomerase IIα (TOP2A), causing double-strand breaks in DNA.[15] These breaks activate key regulators such as p53 and FOXO3 and alter the balance of Bcl-2 family proteins, thereby initiating caspase-dependent apoptosis.[16] Although this DNA damage response is central to its anticancer effect, it also underlies doxorubicin's toxicity.[17] Additional pathways involved in doxorubicin-induced cytotoxicity comprise DNA adduct generation, disruption of DNA and RNA synthesis, and mitochondrial reactive oxygen species (ROS) formation.[18,19] Multiple mechanisms are responsible for doxorubicin resistance, including the activation of drug efflux transporters, alterations in its ability to induce DNA double-strand breaks, and changes in apoptosis signaling. Resistance to doxorubicin in tumor cells is one of the main reasons for clinical treatment failure and cancer relapse.[20] While combinations of doxorubicin with other agents may enhance its efficacy, they may also reduce its cytotoxicity.

Due to the limitations of surgical approaches in all patients and the development of drug resistance in systemic chemotherapy, novel approaches are being explored for HCC treatment. Therefore, we aimed to investigate whether reserpine, previously shown to have anticancer activity in various cancers, enhances the efficacy of doxorubicin in HCC cells under *in vitro* conditions.

MATERIALS AND METHODS

Cell Culture

For this study, HepG2 human HCC cells (ATCC, USA), which can metabolize a variety of nutrients, metabolites, and xenobiotics, were employed. These cells were grown in Dulbecco's Modified Eagle Medium, supplemented with 10% (v/v) heat-inactivated fetal bovine serum, L-glutamine, and antibiotic mix (all cell culture chemicals purchased from Biochrom AG, Germany), and maintained at 37°C in a 5% CO_2 humidified environment (Heraeus, Hanau, Germany). Untreated cells served as the control group. All experiments were carried out in triplicate.[22]

Determination of Cytotoxic Drug Doses (IC_{50})

To determine the cytotoxic effects of reserpine, the MTT [3-(4.5-dimethyl-2-thiazol-2-yl)-2.5-diphenyl-2H-tetrazolium bromide] assay, a quantitative colorimetric method, was used. Following IC_{50} determination, cells were treated with reserpine alone and in combination with doxorubicin for 48 hours. The IC_{50} concentration of doxorubicin in HepG2 cells had previously been reported to be 2 µM.[22]

Assessment of Genotoxic Damage Using the Comet Assay (Single Cell Gel Electrophoresis; SCGE**)**

KÜ Tıp Fak Derg 2025;27(2):192-196
Doi: 10.24938/kutfd.1693317

To evaluate the genotoxic effects of chemical treatments, the alkaline single-cell gel electrophoresis method was employed. Briefly, trypsinized cells were suspended in phosphate-buffered saline, and 5 µL of the cell suspension was combined with 35 µL of low melting point agarose [(1% (w/v)] (LMPA; Sigma-Aldrich), which was then layered onto slides previously coated with 0.5% (w/v) normal melting point agarose (NMPA; Sigma-Aldrich). The slides were placed on ice packs until the agarose solidified. Once the slides were covered with 40 µL of LMPA, they were incubated for 2 hours in a lysis solution (2.5 M NaCl, 100 mM disodium EDTA, 10 mM Tris; pH 10) at +4 ^{0}C. Following this, the slides were incubated in the dark for 20 minutes, in electrophoresis buffer (300 mM NaOH, 1 mM disodium EDTA; pH>13), followed by electrophoresis at 24 V (300 mA) for 30 minutes. After neutralization with 0.4 M Tris (pH 7.5), the slides were stained with EtBr (2µg/mL) and visualized under a fluorescence microscope (Nikon Eclipse 600, Japan). At least two SCGE slides per sample were prepared, and 300 nuclei were scored visually. The nuclei were scored as 0, 1+, 2+, 3+, and 4+ based on the DNA content in the tail and head, as shown in Figure 1. The comet scores were expressed in arbitrary units (AU), calculated as the sum of multiplied scores.[23]

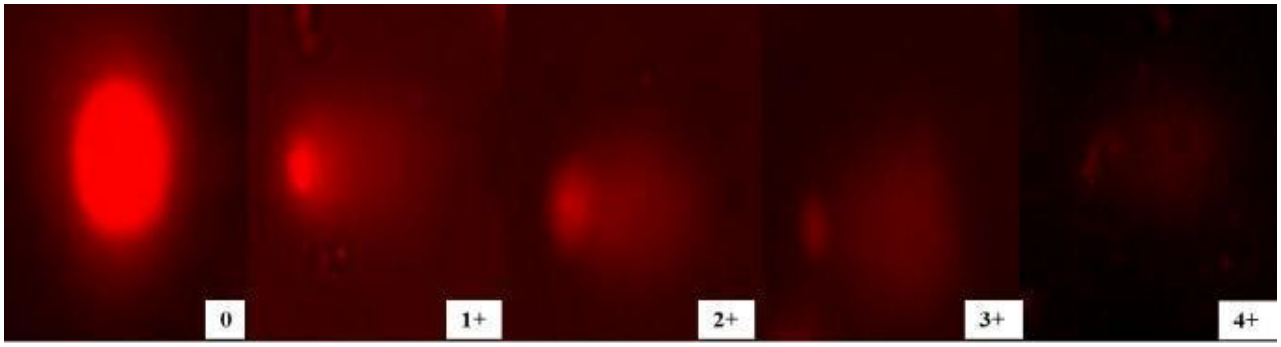

Figure 1: Representative comet images of cells.

Statistical analysis

All statistical analysis was performed using SPSS 11.5 Software (SPSS Inc., USA). The normality of the data was checked using the Shapiro-Wilk test, while Levene's test was used to assess the homogeneity of variances. When data followed a normal distribution and variances were homogeneous, one-way ANOVA was used at a significance level of 0.05, and *post hoc* Tukey tests were conducted to identify groups with significant mean differences. If the data did not meet the assumptions of normality or homogeneity of variance, Mann-Whitney U or Kruskal-Wallis tests, followed by post hoc Dunn's test, were applied at the 0.05 level to compare incubation periods and treatments.

RESULTS

Cytotoxicity Analysis (IC50 Values)

According to the MTT analysis, the IC50 concentration of reserpine in HepG2 cells was determined to be 167.13 µM.

Genotoxicity Analysis (Comet Assay)

The comet scores resulting from the treatments of reserpine and doxorubicin in HCC cells are shown in Table 1. Both reserpine and doxorubicin, when applied alone, significantly induced genotoxic damage compared to the control group ($p<0.05$). Doxorubicin alone caused significantly more genotoxic damage than reserpine alone ($p<0.05$). The combined treatment of doxorubicin and reserpine resulted in significantly higher genotoxic damage than either drug alone ($p<0.05$).

Table 1: Comet assay scores for each treatment group

Group	Comet Score (AU)
Control	18^{ab}
Doxorubicin	185^{ab}
Reserpine	55^{bc}
Doxorubicin + Reserpine	215^{ac}

Statistically significant differences between groups ($p<0.05$) are denoted by superscript letters.

DISCUSSION

Treatment options for patients diagnosed with hepatocellular carcinoma include systemic therapies (e.g., radiotherapy and chemotherapy) and organ transplantation. However, resistance to systemic chemotherapeutic agents, the limited applicability of these treatments to all patients, and complications associated with liver transplantation make HCC treatment challenging. Consequently, new therapeutic approaches for HCC are being actively investigated.[13] One promising approach in HCC treatment is the targeted delivery of cytotoxic chemotherapeutic agents directly to the tumor. Doxorubicin, used in transarterial chemoembolization (TACE), is frequently employed for patients with intermediate-stage HCC and offers a survival benefit. However, numerous factors limit doxorubicin's therapeutic efficacy.[20] To enhance

KÜ Tıp Fak Derg 2025;27(2):192-196
Doi: 10.24938/kutfd.1693317

its effectiveness, doxorubicin has been combined with various drugs and phytochemicals. Among these, studies involving metformin,[24] curcumin,[25] and quercetin[26] are particularly noteworthy.

In our study, we evaluated the effects of reserpine-previously shown to have anticancer activity in various cancers-used alone and in combination with doxorubicin, a chemotherapeutic agent used in HCC treatment. According to our results, reserpine alone induced a low but statistically significant level of genotoxicity; however, in combination with doxorubicin, it increased the genotoxic effects of the drug.

Several mechanisms may contribute to reserpine's augmentation of anticancer medication effectiveness. The initial aspect pertains to the cellular uptake of drugs. Previous studies have shown that in various cancer cells, reserpine potentiates the intracellular accumulation of different anticancer agents, including doxorubicin.[27-29] Secondly, reserpine induces apoptosis in cancer cells by disrupting the cell cycle, inhibiting DNA synthesis, impairing mitochondrial membrane potential, and increasing ROS production.[30,31] A third mechanism involves the modulation of cell signaling pathways. Abnormalities in the β-catenin signaling pathway play a critical role in cancer development. Reserpine and its derivatives suppress β-catenin signaling in various cancer cells, thereby reducing cell proliferation.[32] Another important pathway in cancer development is the TGF-β signaling pathway. Defects in Smad signaling can lead to TGF-β resistance, contributing to uncontrolled cell growth.[33] One study showed that reserpine inhibits TGF–β–dependent phosphorylation of Smad2/3/4, blocks Smad3/Snail activation, and prevents the nuclear translocation of Smad2/4. This leads to the inhibition of DNA repair genes and promotes apoptosis.[2]

We believe that the data obtained in this preliminary study will guide future research and support further investigation into the use of reserpine, currently not actively used in HCC treatment, as an adjuvant agent to enhance the genotoxicity of established anticancer drugs.

This preliminary study demonstrates that reserpine, a naturally occurring alkaloid with known pharmacological effects, enhances the genotoxic potential of doxorubicin in HepG2 hepatocellular carcinoma cells. While reserpine alone exerted a modest genotoxic effect, its co-administration with doxorubicin significantly increased DNA damage, suggesting a synergistic interaction. These findings indicate that reserpine may serve as a potential adjuvant agent to improve the efficacy of conventional chemotherapy in HCC treatment. Further in-depth studies, including mechanistic investigations and *in vivo* evaluations, are warranted to fully understand the therapeutic potential and clinical applicability of this combination strategy.

Conflict of Interest: The authors declared no conflicts of interest.

Support and Acknowledgments: We would like to thank Cevriye Yıldırım and Hazal Ceylan for their technical assistance.

Researchers' Contribution Rate Statement: Concept/Design: SÖ, SNŞ, ES, BNB, SBA, ZB, EY; Analysis/Interpretation: SÖ, SNŞ, ES, BNB, SBA, ZB, EY; Data Collection: SÖ, SNŞ, ES, BNB, SBA, ZB, EY; Writer: SÖ, SNŞ, ES, BNB, SBA, ZB, EY; Critical Review: SÖ, SNŞ, ES, BNB, SBA, ZB, EY; Approver: SÖ, SNŞ, ES, BNB, SBA, ZB, EY

Support and Acknowledgment: No financial support was received from any institution or person.

REFERENCES

1. Al-Bloushi S, Safer AM, Afzal M, Mousa SA. Green tea modulates reserpine toxicity in animal models. *J Toxicol Sci*. 2009;34(1):77-87.
2. Ramu AK, Ali D, Alarifi S, Syed Abuthakir MH, Ahmed Abdul BA. Reserpine inhibits DNA repair, cell proliferation, invasion and induces apoptosis in oral carcinogenesis via modulation of TGF-β signaling. *Life Sci*. 2021;264:118730.
3. Abdelfatah SAA, Efferth T. Cytotoxicity of the indole alkaloid reserpine from *Rauwolfia serpentina* against drug-resistant tumor cells. *Phytomedicine*. 2015;22(2):308-318.
4. Scaggiante B, Kazemi M, Pozzato G et al. Novel hepatocellular carcinoma molecules with prognostic and therapeutic potentials. *World J Gastroenterol*. 2014;20(5):1268-1288.
5. Bray F, Laversanne M, Sung H et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2024;74(3):229-263.
6. Aravalli RN, Cressman ENK, Steer CJ. Cellular and molecular mechanisms of hepatocellular carcinoma: An update. *Arch Toxicol*. 2013;87(2):227-247.
7. Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: From genes to environment. *Nat Rev Cancer*. 2006;6(9):674-687.
8. Hoshida Y, Toffanin S, Lachenmayer A, Villanueva A, Minguez B, Llovet JM. Molecular classification and novel targets in hepatocellular carcinoma: Recent advancements. *Semin Liver Dis*. 2010;30(1):35-51.
9. Llovet JM, Villanueva A, Lachenmayer A, Finn RS. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. *Nat Rev Clin Oncol*. 2015;12(8):436.
10. Schulze K, Imbeaud S, Letouzé E et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet*. 2015;47(5):505-511.
11. Zehir A, Benayed R, Shah RH et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*. 2017;23(6):703-713.
12. Chidambaranathan-Reghupaty S, Fisher PB, Sarkar D. Hepatocellular carcinoma (HCC): Epidemiology, etiology and molecular classification. *Adv Cancer Res*. 2021;149:1-61.

13. Vogel A, Meyer T, Sapisochin G, Salem R, Saborowski A. Hepatocellular carcinoma. *Lancet*. 2022;400(10360):1345-1362.
14. Ramamoorthy MD, Kumar A, Ayyavu M, Dhiraviam KN. Reserpine induces apoptosis and cell cycle arrest in hormone independent prostate cancer cells through mitochondrial membrane potential failure. *Anticancer Agents Med Chem*. 2018;18(9):1313-1322.
15. Tewey KM, Rowe TC, Yang L, Halligan BD, Liu LF. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science*. 1984;226(4673):466-468.
16. Roos WP, Kaina B. DNA damage-induced cell death: From specific DNA lesions to the DNA damage response and apoptosis. *Cancer Lett*. 2013;332(2):237-248.
17. Anti-apoptotic mechanisms of drug resistance in cancer - PubMed. Accessed April 30, 2025. https://pubmed.ncbi.nlm.nih.gov/19442051/
18. Swift LP, Rephaeli A, Nudelman A, Phillips DR, Cutts SM. Doxorubicin-DNA adducts induce a non-topoisomerase II-mediated form of cell death. *Cancer Res*. 2006;66(9):4863-4871.
19. Gewirtz DA. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem Pharmacol*. 1999;57(7):727-741.
20. Cox J, Weinman S. Mechanisms of doxorubicin resistance in hepatocellular carcinoma. *Hepat Oncol*. 2016;3(1):57-59.
21. O'Brien NM, Woods JA, Aherne SA, O'Callaghan YC. Cytotoxicity, genotoxicity and oxidative reactions in cell-culture models: Modulatory effects of phytochemicals. *Biochem Soc Trans*. 2000;28(2):22-26.
22. Yurtcu E, İşeri Ö, Sahin F. Genotoxic and cytotoxic effects of doxorubicin and silymarin on human hepatocellular carcinoma cells. *Hum Exp Toxicol*. 2014;33(12):1269-1276.
23. Akkaş H, Aydın E, Babakurban S, Yurtcu E, Özbek Ö. Effect of mometasone furoate nasal spray on the DNA of nasal mucosal cells. *Turkish Journal of Medical Sciences*. 2018;48(2):339-345.
24. Vallianou NG, Evangelopoulos A, Kazazis C. Metformin and cancer. *Rev Diabet Stud*. 2013;10(4):228-235.
25. Nagamine M, Sadzuka Y, Sonobe T. Antitumor activity of doxorubicin is enhanced by curcumin. *Cancer Research*. 2006;66(8_Supplement):503.
26. Liu S, Li R, Qian J et al. Combination therapy of doxorubicin and quercetin on multidrug-resistant breast cancer and their sequential delivery by reduction-sensitive hyaluronic acid-based conjugate/d-α-tocopheryl poly(ethylene glycol) 1000 succinate mixed micelles. *Mol Pharm*. 2020;17(4):1415-1427.
27. Yoshida T, Shimizu K, Ushio Y, Mogami H, Sakamoto Y. Modulation *in vitro* and *in vivo* of ACNU resistance in a subline of C6 glioma with reserpine. *J Neurosurg*. 1987;66(2):251-255.
28. Chitnis MP, Kamath NS, Advani SH. Mitoxantrone & adriamycin cytotoxicity enhanced by reserpine in human chronic myeloid leukaemia cells. *Indian J Med Res*. 1989;90:197-202.
29. Viladkar A, Chitnis M. Sensitization of P388 murine leukemia cells to epirubicin cytotoxicity by reserpine. *Cancer Biother*. 1993;8(1):77-85.
30. Ramamoorthy MD, Kumar A, Ayyavu M, Dhiraviam KN. Reserpine induces apoptosis and cell cycle arrest in hormone independent prostate cancer cells through mitochondrial membrane potential failure. *Anticancer Agents Med Chem*. 2018;18(9):1313-1322.
31. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Res*. 1995;22(4):375-383.
32. Gwak J, Song T, Song JY et al. Isoreserpine promotes beta-catenin degradation via Siah-1 up-regulation in HCT116 colon cancer cells. *Biochem Biophys Res Commun*. 2009;387(3):444-449.
33. Samanta D, Datta PK. Alterations in the Smad pathway in human cancers. *Front Biosci (Landmark Ed)*. 2012;17(4):1281-1293.