Available Online at

http://www.ijcpa.in

July-September 2021

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726; pISSN: 2395-2466

DOI: http://dx.doi.org/10.21276/ijcpa

Research Article | Volume-8 | Issue-4 | Article ID: 0067

ANTI-CANCER EFFECTS OF GRAPE SEED ON ISHIKAWA CELL CULTURE

Ozlem Ozgur Gursoy[1], Ceren Yildiz Eren[1*], Hulusi Goktug Gurer[1], Canan Vejselova Sezer[2]

[1]Department of Obstetrics and Gynecology Clinic, Eskisehir Acibadem Hospital, Eskisehir, Turkey

[2]Department of Biology, Eskişehir Technical University, Eskisehir, Turkey

***Corresponding Author:** *E-mail:* **cyeren@yaani.com**

Received date: 27 August 2021; ***Accepted date:*** 10 September 2021; ***Published date:*** 17 September 2021

ABSTRACT

Cancer is a serious health problem. It still affects millions of people. In vitro cell culture is a method used to study the behavior of cells. The interest in grape seed is due to its high content of antioxidants in the form of proanthocyanidins. Its antioxidant capacity is greater than known antioxidants.

Ishikawa cells were cultured in complete DMEM/F-12 medium containing 100 units/mL-100 µg/mL of penicillin-streptomycin and 10% fetal bovine serum in 37 ℃ and 5% CO_2 incubator. After incubation of Ishikawa cells MTT (5 mg/mL) dye was added (20 µL/well) and plates were incubated again for two hours. Cell death mode triggered by grape seed extract on Ishikawa cells was analyzed by using the Annexin-V technique. To test the activation of caspases on Ishikawa cells exposed to grape seed extract Caspase 3/7 technique was used. For analysis of results one way variance analysis for multiple comparisons of GraphPad Prism 6.0 for Windows was used.

Grape seed extract showed its toxicity on Ishikawa cells in dose dependent manner in 24 and 48 hours of exposure. Annexin-V technique showed that grape seed extract induced apoptosis on Ishikawa cells. When compared to the control group. Ishikawa cells treated with grape seed extract imply to total apoptosis percentage of 22.90%. Based on this finding it can be concluded that grape seed extract has apoptosis inducing activity on Ishikawa cells in short application time.

Thus, grape seed extract worth to be elucidated for its further anticancer effects with deeper mechanistic studies in order to provide an option for designing novel anticancer drug as supplement or alternative to current chemotherapeutics.

Key Words-*Grape seed extract, Ishikawa cell culture, Anti-cancer effects, MTT assay, Annexin-V, Caspase*

INTRODUCTION

Cancer is a serious health problem. It still affects millions of people around the world. It is a disease in which certain cells in the body grow uncontrollably and spread to other parts of the body. It can start in almost any part of the human body. Endometrial cancer is the most common type of cancer in developed countries and the second most common type of cancer in developing countries. Five-year survival of patients with late-diagnosed endometrial cancer is below 30%. Therefore, it is very important to develop an appropriate

chemotherapeutic regimen for late-stage endometrial cancer[1].

In vitro cell culture is a method used to study the behavior of cells. A controlled environment is created in cell culture and this environment is protected from possible variations. Today, a wide variety of cell cultures have been developed. Cell cultures are used in areas such as basic cell biology, the effects of drugs and other chemicals, and vaccine production. The *in vitro* study of cellular activity has several advantages and disadvantages. The most important advantage of cell cultures is the consistency and reproducibility of the results obtained. In addition, cell cultures are working environments where physiological conditions can be precisely controlled. In these environments, the results are pretty clear. Although it is accepted that cell cultures do not fully represent processes in living tissue, it is currently considered the most precise and controlled method for research projects. The use of cell cultures in research is the first choice in areas such as the study of pathophysiology and therapeutic targets of various diseases and disorders[2,3].

MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. For cell populations, total mitochondrial activity is related to the number of viable cells. Therefore, MTT is widely used to measure the *in vitro* cytotoxic effects of drugs on cell lines or diseased cells[4,5].

Mitochondria play a central role in apoptosis. Apoptosis is programmed cell death associated with caspase enzyme activity to maintain homeostasis in multicellular organisms. It is one of the most important intracellular events in the living cell. Caspase-3 and caspase-7 are cysteine-aspartic acid proteases that can directly perform apoptosis after caspase activation. Imaging of apoptosis in living cells provides great advantages, especially in drug research. One of the biochemical features of cells undergoing apoptosis is loss of plasma membrane asymmetry. As a result of this loss, a high amount of phosphatidylserine (PS) is released on the outer cell surface. Aennexin V is a phospholipid-binding protein with a high affinity for PS and binds tightly to PS. Therefore, fluorescently labeled Annexin V can be used to detect PS released in apoptotic cells[6-8].

A wide variety of studies over the years have identified the presence of potential agents in routinely consumed plant-based diets. These are mostly phytochemicals such as non-nutritive phenolics, glucosinolates, and terpenoids, which are divided into different classes according to their chemical structure. They are present in fruits, vegetables, nuts, and commonly consumed beverages such as tea and coffee. These phytochemicals show selective toxicity against cancerous or pre-cancerous cells. They have aroused scientific interest because they meet the basic requirements of an ideal chemopreventive agent, such as efficacy, oral route of administration, and acceptance by the target human population[9].

Grape seed is an over-the-counter product used to contribute to a healthy life, widely consumed as a food supplement. The interest in grape seed is due to its high content of antioxidants in the form of proanthocyanidins. Its antioxidant capacity is greater than known antioxidants such as vitamin C and E[10].

MATERIALS AND METHODS

Human endometrial adenocarcinoma Ishikawa cells were obtained from the American Type Culture Collection (Manassas, USA). Grape seed extract was obtained from Akcan Kimya (Turkey), fetal bovine serum (FBS), penicillin/streptomycin, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-2H-tetrazolium bromide (MTT), and Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) were purchased from Sigma-Aldrich (St. Louis, USA). Caspase 3/7 and Annexin-V Kits were from (Merck, Millipore, USA).

Culture of ishikawa Cells

Ishikawa cells were cultured in complete DMEM/F-12 medium containing 100 units/mL-100 µg/mL of penicillin-streptomycin and 10% fetal bovine serum in 37 °C and 5% CO_2 incubator. The test cells with confluency of 85% were passaged routinely per 3 days and used for experimentations.

MTT assay

Grape seed extract was diluted in DMSO to obtain a stock solution for further dilutions during the experimentation. The stock solution of grape seed extract was diluted with freshly prepared complete DMEM/F-12 medium and placed in 96 well test plates. Ishikawa cells were seeded (5×10^3/well) in 96 well culture plates and incubated for 24 and 48 hours in 37 °C and 5% CO_2 incubator conditions. After incubation MTT (5 mg/mL) dye was added (20 µL/well) and plates were incubated again for 2 hours. After the second incubation, all of the solutions were aspirated from the plates and DMSO (200 µL/well) was added to dissolve the formazan crystals. Absorbances of the plates were read at 570 nm (n=3) on an ELISA reader (HTX Synergy, BioTek, USA). Obtained values were used for calculating the viability percentages and IC_{50} values.

Annexin-V assessment

Cell death mode triggered by grape seed extract on Ishikawa cells was analyzed by using the Annexin-V technique. In this manner, Ishikawa cells were plated in six-well culture plates (5×10^5 cells/well) and exposed to IC_{50} dose of grape seed extract for 24 hours at 37 °C and 5% CO_2 incubator conditions. All cell samples were trypsinized and harvested in separate test tubes. Collected cells were washed in buffer (PBS). Annexin-V dye (100 µL/sample) was added and further incubated for 20 minutes at dark at room temperature (Muse® Annexin-V and Dead Cell Assay Kit). Ishikawa cells were analyzed with Muse™ Cell Analyzer (Merck, Millipore, Hayward, California, USA).

Caspase 3/7 activation assessment

To test the activation of caspases on Ishikawa cells exposed to grape seed extract Caspase 3/7 technique was used. Ishikawa cells exposed to IC_{50} value of grape seed extract for 24 hours in six-well plates and untreated Ishikawa cells cultured in the same conditions and density (5×10^5/well with test cells were harvested by trypsinization and washed in PBS. Caspase 3/7 working solution and 7-ADD solutions were added to all sample groups according to the user manual of the manufacturer of the caspase 3/7 kit (Merck, Millipore, Hayward, California, USA). Samples were analyzed on a cell analyzer (Muse TM Cell Analyzer, Merck, Millipore, Hayward, California, USA).

Statistical analysis

For analysis of results one way variance analysis for multiple comparisons of GraphPad Prism 6.0 for Windows was used.

RESULTS

MTT findings

Grape seed extract showed its toxicity on Ishikawa cells in dose dependent manner in 24 and 48 hours of exposure (Figure 1). The highest decrease was detected at 1 mg/mL of grapes seed extract both for 24 and 48 hours of application. IC_{50} values of grape seed extract were detected to be 0.09 mg/mL for both exposure times. Moreover, the obtained data imply to the efficacy of grape seed extract in growth inhibition and cytotoxicity both for application periods of 24 and 48 hours.

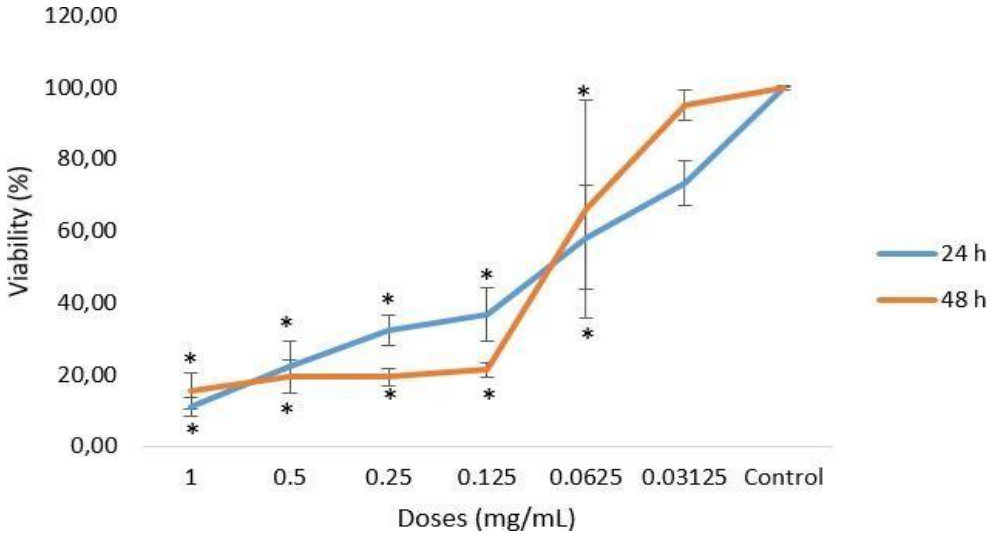
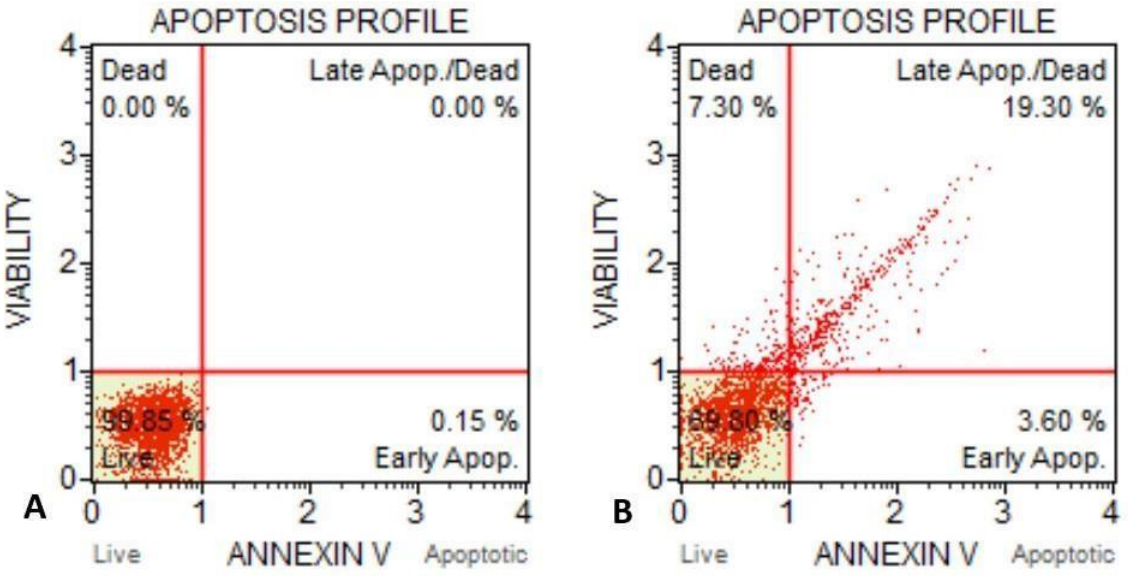


Fig. 1: Growth inhibition activity of grape seed extract on Ishikawa cells. IC_{50} dose of grape seed extract: 0.09 mg/mL for 24 and 48 hours. (*: p<0.5)

Annexin-V results

Apoptotic profiles of ishikawa cells analyzed by annexin-V technique showed that grape seed extract induced apoptosis on Ishikawa cells. When compared to the control group (Figure 2A) Ishikawa cells treated with grape seed extract imply to total apoptosis percentage of 22.90% (Figure 2B). Based on this finding it can be concluded that grape seed extract has apoptosis inducing activity on Ishikawa cells in short application time.



. Fig. 2: Apoptosis profiles of Ishikawa cells

Note: 2A. Control Ishkiawa cells; Live cells 99.85% and 0.15% early apoptotic cells were detected. Percentages of late apoptotic and dead cells were detected as 0.00%.2B. Ishikawa cells exposed to IC_{50} value of grapes eed extract for 24 hours. 69.80% of cells were live. The percentages of dead, early apoptotic and late apoptotic cells were detected to be 7.30%, 3.60% and 19.30% respectively

Caspase 3/7 results

Cells undergo apoptosis *via* various pathways and by the influence of intracellular or extracellular factors. Caspases are major elements of those apoptotic pathways that initiate apoptosis irreversibly by their activation. Herein, the total percentage of activated caspases was found to be 6.50%. In control cells this percentage was detected as 3.05% (Figure 3). Flow cytometry findings underlined that grape seed extract induced apoptotic cell death in Ishikawa cells and based on the caspase 3/7 analysis results it was clearly detected that the triggered apoptosis is caspase-dependent.

Based on the data obtained within the scope of this study it is concluded that grape seed extract exhorts cytotoxicity on human endometrial adenocarcinoma cells in low doses being anti-proliferative and proapoptotic in dose-dependent manner. Thus, grape seed extract worth to be elucidated for its further anticancer effects with deeper mechanistic studies in order to provide an option for designing novel anticancer drug as supplement or alternative to current chemotherapeutics.

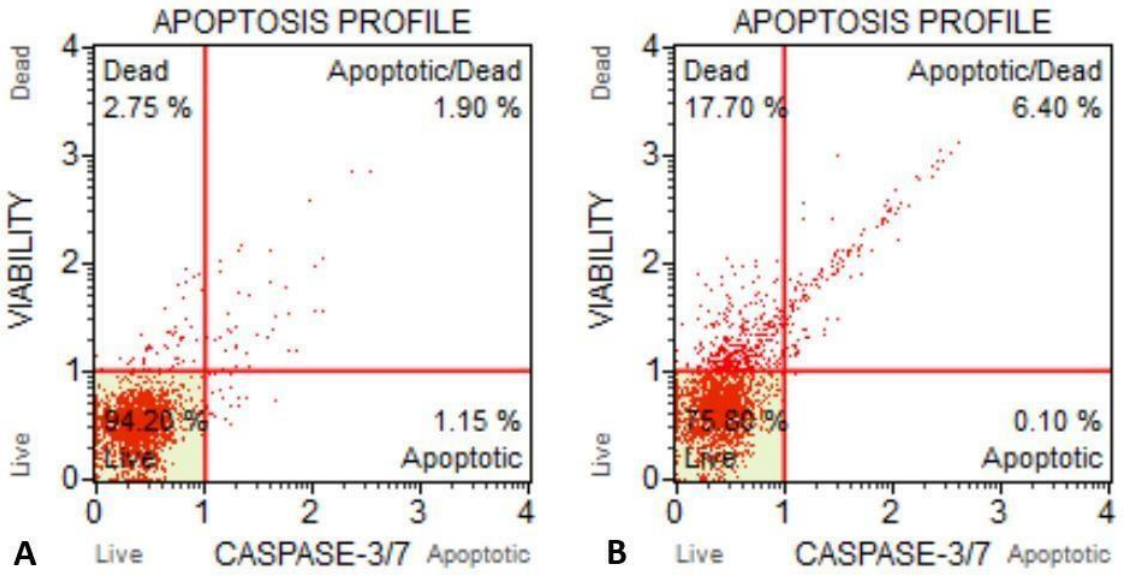


Fig. 3: Caspase-dependent apoptosis profiles of Ishikawa cells

Note: 3A. Untreated Ishikawa cells (94.20% Live, 2.75% Dead, 1.90 Apoptotic/Dead and 1.15% Apoptotic cells). 3B. Ishikawa cells treated with IC_{50} concentration of grape seed extract for 24 hours (75.80% Live, 17.7% Dead, 6.4% Apoptotic/Dead and 0.10% apoptotic cells)

DISCUSSION

Grape seed extract is a mixture of various compounds of polyphenols and phenolic acids. Their consumption is safe. It is considered to have health benefits. The extract-derived anti-tumor activity acts through a variety of biological mechanisms and cellular targets. It causes inhibition of cell growth and apoptosis in various cancer cell lines. These effects are modulated at the molecular level. Anti-cancer activity from grape seed extract is mostly based on the increase of reactive oxygen species. This increase is followed by the upregulation of several key molecular pathways, including kinases, nuclear factor kappa B, cytoskeletal proteins, and metalloproteinases. Promising results have been obtained on grape seed extract *in vitro* as well as in animal studies. It is thought to be a source of potential new pharmacological molecules and an important opportunity for clinical research [11–13].

Flavonoids contain various classes of compounds such as flavones, flavonols, and anthocyanins. Each class is different from the other. Flavonoids are generally found in nature as glycosides. Grape seed has both phenolic acid and flavonoid content. This content constitutes 60%-70% of the dry extract. Grape seeds also contain resveratrol and anthocyanidin, but their density is less1[14,15].

It has been shown that grape seed extract components have antioxidant, anti-inflammatory, antiaggregant and antimicrobial effects. These properties are attributed specifically to more potent ingredients such as gallic acid, procyanidins and quercetin. Gallic acid and procyanidins generally make up about 80% of the dry extract. The effects of grape seed extract are often attributed to these molecules. However, since some functions are provided by synergistic interactions between different components, the contribution of other molecules cannot be ignored. For example, the antioxidant effects of the extract can be explained by the sum of the antioxidant activities of each component. Similarly, even though it is thought that the anticancer effects are generally performed by procyanidins and epigallo-catechin-3-gallate, the anticancer effect is higher than the sum of each component[13-16].

In this case, the effect obtained from grape seed extract is greater than the effects of the components contained in the extract alone. This indicates that there is a synergism between the components. In this respect, it is more meaningful to examine the grape seed extract as a whole when examining its health effects, and the results will be more reliable. Considering this issue, grape seed extract was used as a whole in our research.

When the data of some studies are examined, it is seen that important results have been obtained. The PI3K/Akt pathway plays a crucial role in mammalian cell survival signals and has been shown to be activated in various cancers. Phosphorylated PI3K and Akt are thought to be key elements in modulating kinase activation and NF-kB-dependent pathways. Grape seed extract has been shown to

reduce PI3K levels and Akt phosphorylation, increasing Akt degradation. PI3K/Akt phosphorylation is negatively regulated by 'chromosome 10 deleted phosphatase and tensin homolog' (PTEN), a lipid phosphatase that inhibits it. The absence of PTEN is strongly associated with PI3K/Akt activation in tumor cell lines. Grape seed extract significantly reduces PTEN phosphorylation and increases its negative regulation on the PI3-K pathway. In addition, grape seed extract suppresses Akt-related effects on CREB, NFkB, BAD and Bcl-2, promoting a general pro-apoptotic effect on cancer cells[17–20].

Mitogen activated protein kinase (MAPK) signaling pathway is an important regulator of transcriptional factor activities. It affects various extracellular stimuli by controlling the activities of MAPK transcription factors and plays a role in cancer development and progression. It has been reported by some studies that grape seed extract increases the activation of JNK and p38MAPK through increased intracellular calcium. In contrast, p38MAPK increases apoptosis through Bcl-2 inactivation, caspase increase and mitochondria depolarization. This effect of p38MAPK has been associated with an increase in intracellular calcium and is thought to participate in the enhancement of grape seed extract-induced apoptotic effect on cancer cells[21–24].

Some studies have shown that inflammation modulators are functionally associated with tumor development. Prostaglandins, one of the most important of these modulators, are produced in abundance by the metabolic conversion of arachidonic acid by COX-2. Prostaglandins produced are known to play a role in the development of many malignancies. NF-kB increases COX-2 expression. Grape seed extract has been proven to down-regulate the expression of NF-kB and COX-2. Activator protein-1 (AP-1) levels, which increase COX-2 expression, are also down-regulated by grape seed extract. Data from various studies show that grape seed extract inhibits cancer development by reducing the level of prostaglandins[25-28].

Data have been reported proving that grape seed extract induces apoptosis in cancer cells by down-regulating anti-apoptotic proteins. These effects are due to inhibition of the PI3K pathway and modulation of p38MAPK and ERK, as mentioned above. This pro-apoptotic effect is highly specific, as normal cells are generally insensitive to grape seed extract. It has been reported that grape seed extract induces apoptosis in p53-expressing cancer cells more than others. Some components in the extract recognize p53 as a specific target and bind to integrin αvβ3, leading to p53 activation. However, there are also studies showing that the cytotoxic effect of grape seed extract is independent of the p53 status of cancer cell lines. One of the most common genetic defects found in cancers is the mutation of the TP53 gene, which encodes the p53 protein. Therefore, the observation that the cytotoxic effect is independent of p53 status is very important[29-32].

CONCLUSION

In conclusion, the data obtained in our research is supported by many studies. It is thought that it is important to reveal the effect shown on proteins such as caspases and annexin in our study and to support this with MTT analysis. The fact that the grape seed extract was examined as a whole in our study is also important in terms of revealing the synergistic effects of the components it contains. It is considered that the comparative examination of seed extracts obtained from different grape species will contribute to the subject in the following studies.

REFERENCES

1. Devis-Jauregui L, Eritja N, Davis ML, Matias-Guiu X, Llobet-Navàs D. Autophagy in the physiological endometrium and cancer. Autophagy. 202;17(5):1077-1095.
2. Katt ME, Placone AL, Wong AD, Xu ZS, Searson PC. In vitro tumor models: advantages, disadvantages, variables, and selecting the right platform. Frontiers in bioengineering and biotechnology. 2016;4:12.
3. Namekawa T, Ikeda K, Horie-Inoue K, Inoue S. Application of prostate cancer models for preclinical study: advantages and limitations of cell lines, patient-derived xenografts, and three-dimensional culture of patient-derived cells. Cells.2019 ;8(1):1-27.
4. Grela E, Kozłowska J, Grabowiecka A. Current methodology of MTT assay in bacteria–A review. Acta histochemica. 2018;120(4):303-311.
5. Moscheni C, Malucelli E, Castiglioni S, Procopio A, De Palma C, Sorrentino A, et al. 3D quantitative and ultrastructural analysis of mitochondria in a model of doxorubicin sensitive and resistant human colon carcinoma cells. Cancers. 2019;11(9):2-15.
6. Hammill AK, Uhr JW, Scheuermann RH. Annexin V staining due to loss of membrane asymmetry can be reversible and precede commitment to apoptotic death. Experimental cell research. 1999;251 (1):16-21.
7. Li J, Yuan J. Caspases in apoptosis and beyond. Oncogene. 2008 ;27(48):6194-6206.
8. Shim MK, Yoon HY, Lee S, Jo MK, Park J, Kim JH, et al. Caspase-3/-7-specific metabolic precursor for bioorthogonal tracking of tumor apoptosis. Scientific reports. 2017;7(1):1-5.

9. Galati G, O'brien PJ. Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free Radical Biology and Medicine. 2004 ;37(3):287-303.
10. Kaur M, Agarwal C, Agarwal R. Anticancer and cancer chemopreventive potential of grape seed extract and other grape-based products. The Journal of nutrition. 2009;139(9):1806S-1812S.
11. Hughes RJ, Croley TR, Metcalfe CD, March RE. A tandem mass spectrometric study of selected characteristic flavonoids. International Journal of Mass Spectrometry. 2001;210:371-85.
12. Iacopini P, Baldi M, Storchi P, Sebastiani L. Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, in vitro antioxidant activity and interactions. Journal of Food Composition and Analysis. 2008;21(8):589-598.
13. Dinicola S, Cucina A, Antonacci D, Bizzarri M. Anticancer effects of grape seed extract on human cancers: a review. Journal of Carcinogenesis and Mutagenesis. 2014;8(005):2-14
14. Veluri R, Singh RP, Liu Z, Thompson JA, Agarwal R, Agarwal C. Fractionation of grape seed extract and identification of gallic acid as one of the major active constituents causing growth inhibition and apoptotic death of DU145 human prostate carcinoma cells. Carcinogenesis. 2006;27(7):1445-1453.
15. Pan MH, Chiou YS, Wang YJ, Ho CT, Lin JK. Multistage carcinogenesis process as molecular targets in cancer chemoprevention by epicatechin-3-gallate. Food & function. 2011;2(2):101-110.
16. Apostolou A, Stagos D, Galitsiou E, Spyrou A, Haroutounian S, Portesis N, et al. Assessment of polyphenolic content, antioxidant activity, protection against ROS-induced DNA damage and anticancer activity of Vitis vinifera stem extracts. Food and Chemical Toxicology. 2013;61:60-68.
17. Engelbrecht AM, Mattheyse M, Ellis B, Loos B, Thomas M, Smith R, et al. Proanthocyanidin from grape seeds inactivates the PI3-kinase/PKB pathway and induces apoptosis in a colon cancer cell line. Cancer letters. 2007;258(1):144-153.
18. Qin J, Xie LP, Zheng XY, Wang YB, Bai Y, Shen HF, et al. A component of green tea,(−)-epigallocatechin-3-gallate, promotes apoptosis in T24 human bladder cancer cells via modulation of the PI3K/Akt pathway and Bcl-2 family proteins. Biochemical and biophysical research communications. 2007;354(4):852-857.
19. Dolcet X, Llobet D, Pallares J, Matias-Guiu X. NF-kB in development and progression of human cancer. Virchows archiv. 2005;446(5):475-482.
20. Chang F, Lee JT, Navolanic PM, Steelman LS, Shelton JG, Blalock WL, et al. Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. Leukemia. 2003;17(3):590-603.
21. Dinicola S, Mariggio MA, Morabito C, Guarnieri S, Cucina A, Pasqualato A, et al. Grape seed extract triggers apoptosis in Caco-2 human colon cancer cells through reactive oxygen species and calcium increase: extracellular signal-regulated kinase involvement. British Journal of Nutrition.2013; 110(5):797-809.
22. Wagner EF, Nebreda ÁR. Signal integration by JNK and p38 MAPK pathways in cancer development. Nature Reviews Cancer. 2009;9(8):537-549.
23. Hussain AF, Sulaiman GM, Dheeb BI, Hashim AJ, Abd Alrahman ES, Seddiq SH,et al. Histopathological changes and expression of transforming growth factor beta (TGF-β3) in mice exposed to gliotoxin. Journal of King Saud University-Science. 2020 ;32(1):716-725.
24. Chen KC, Liu WH, Kao PH, Chang LS. Calcium-stimulated mitogen-activated protein kinase activation elicits Bcl-xL downregulation and Bak upregulation in notexin-treated human neuroblastoma SK-N-SH cells. Journal of cellular physiology. 2010;222(1):177-186.
25. Gopalakrishnan A, Kong AN. Anticarcinogenesis by dietary phytochemicals: cytoprotection by Nrf2 in normal cells and cytotoxicity by modulation of transcription factors NF-κB and AP-1 in abnormal cancer cells. Food and Chemical Toxicology. 2008;46(4):1257-1270.
26. Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. Nature Reviews Cancer. 2003;3(11):859-868.
27. Kundu JK, Shin YK, Surh YJ. Resveratrol modulates phorbol ester-induced pro-inflammatory signal transduction pathways in mouse skin in vivo: NF-κB and AP-1 as prime targets. Biochemical pharmacology. 2006;72(11):1506-1515.
28. Perkins ND. Integrating cell-signalling pathways with NF-κB and IKK function. Nature reviews Molecular cell biology. 2007;8(1):49-62.
29. Hastak K, Agarwal MK, Mukhtar H, Agarwal ML. Ablation of either p21 or Bax prevents p53-dependent apoptosis induced by green tea polyphenol epigallocatechin-3-gallate. The FASEB journal. 2005;19(7):1-9.
30. Hollstein M, Rice K, Greenblatt MS, Soussi T, Fuchs R, Sørlie T,etal. Database of p53 gene somatic mutations in human tumors and cell lines. Nucleic acids research. 1994;22(17):3551.
31. Manna S, Banerjee S, Mukherjee S, Das S, Panda CK. Epigallocatechin gallate induced apoptosis in Sarcoma180 cells in vivo: mediated by p53 pathway and inhibition in U1B, U4-U6 UsnRNAs expression. Apoptosis. 2006;11(12):2267-2276.

32. Hsu CP, Lin YH, Chou CC, Zhou SP, Hsu YC, Liu CL, et al. Mechanisms of grape seed procyanidin-induced apoptosis in colorectal carcinoma cells. Anticancer research. 2009 ;29(1):283-289.