RESEARCH ARTICLE

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Grape Seed Extract Induces G2/M Cell Cycle Arrest and Apoptosis Via Generation of Reactive Oxygen Species in Breast and Colon Cancer Cell lines

Reem A Yaseen[1*], Aly Fahmy Mohamed[2], Ashraf Bakkar[1], Rania Ibrahim Shebl[3], Jean Marc Sabatseir[4]

Abstract: Grape seed as a fruit waste was found to have medicinal potentials, specially its procyanidine containment. The present work aimed to investigate the anticancer potential against two of the mostly spread cancers worldwide namely breast and colon cancers. Data recorded revealed that grape seed extract (GSE) demonstrated a dose dependent cytotoxic potential with an IC_{50} values of 148.5 and 190.2 µg/ml for CaCo-2 and MCF-7 respectively. Gene expression pattern of apoptosis related genes revealed that GSE stimulates a p53-independent apoptotic pathway which was associated with G2/M phase arrest ($p<0.05$). The elevated level of reactive oxygen species (ROS) in the order of 28.5 % and 40.8 % in CaCo-2 and MCF-7, respectively suggested that GSE is involved also in initiating the intrinsic pathway of apoptosis. These findings focus on the potential of using GSE as a multi-targeted cancer therapy to overcome the problem of resistance to current treatments.

INTRODUCTION

Cancer is known to be one of the most life-threatening diseases worldwide. There are many types of cancer, in different forms and stages; therefore, it is a challenge and a priority to search for a suitable cure. Breast cancer is the second world widespread cancer with an estimated 249, 260 new cases in 2016. Colon cancer is the third most common type of cancers with an approximately 49,190 deaths in both sexes. [1] Chemotherapy is the most used method for treatment of cancer cases. It is very effective method in most cases; however, it has various side effects. The short term side effects of chemotherapy are nausea, fatigue and stomatitis while cardiac dysfunction and infertility are considered of the long term side effects. [2] Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are well-established molecules responsible for the deleterious effects of oxidative stress. Accumulation of free radicals coupled with an increase in oxidative stress has been implicated in the pathogenesis of several disease states. The role of oxidative stress in vascular diseases, diabetes, renal ischemia, atherosclerosis, pulmonary pathological states, inflammatory diseases, cancer, as well as aging has been well established. [2] Free radicals and other reactive species are constantly generated *in-vivo* and cause oxidative damage to biomolecules. In the meantime, their effect is neutralized by the existence of multiple antioxidants and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. [3]

Nowadays researchers discovered some antioxidant components in fruits that exhibit potential anti-cancer activity. Grape *(Vitis vinifera)* is used as a food supplement and a source of treatment because of its beneficial nutritional and medical values. *Vitis vinifera* was also found to have antioxidant components including polyphenols (procyanidine) and phenolic acids which suggest a potential anti-proliferative and anticancer efficacy in addition to anti-inflammatory activities. [4] Accordingly, the present study aimed to evaluate the anti-cancer potential of *Vitis vinifera* seeds derived procyanidine against breast (MCF-7) and colon (CaCO-2) cancer cell lines in relation to apoptotic and cell cycle pattern and ROS as well.

MATERIALS AND METHODS

Cell Culture

Breast (MCF-7) and colon (CaCo-2) cancer cells were kindly supplied obtained from VACSERA culture department. Cells were maintained in RPMI 1640 medium (GIBCO - USA) supplemented with 10% fetal bovine serum (GIBCO - USA) in a humidified atmosphere of 5% CO_2 at 37°C (Jouan - France).

Grape Seed Extract (GSE) Preparation

Grape seed powder was purchased from Sigma - Aldrich, USA and suspended in distilled water as 3.08 µg/ml. The mixture was heated in a water bath 70°C for 30 mins followed by sterilization using 0.22 µm syringe filter (Milipore-USA).

Cytotoxicity

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) purchased from (Sigma Aldrich-USA)is a water soluble tetrazolium salt, which is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase realized from the mitochondria. The formazan product is impermeable to the cell membranes and therefore it accumulates in healthy cells. MTT 0.5 mg/ ml was added to 24 hr GSE treated cells as 50 µl/well. Plates were incubated at 37°C for 4 hr in the dark. MTT stain was decanted and stained cells are washed twice

[1]Biochemistry Department, Faculty of Biotechnology, MSA University, 26 July Mehwar Road Intersection with Wahat Road, 6th October City, Egypt.
E-mail: remayaseen95@gmail.com
*Corresponding author

[2]Holding Company for the Production of Vaccines, Sera and Drugs (VACSERA Egy Vac), 51 Wezaret EL Zeraa Street, Agouza, Giza, Egypt.

[3]Microbiology and Immunology Department, Faculty of Pharmacy, Ahram Canadian University (ACU), 4th Industrial Zone, Banks Complex, 6th of October City, Giza Governorate, Egypt.

[4]INSERM UMR S1097, Aix-Marseille University, Jardin du Pharo, 58 Boulevard Charles Livon, 13284 Marseille, Cedax 07-France.

using phosphate buffer saline (PBS) solution (Biowhittaker - Belgium). Developed formazan crystals were dissolved using 0.4% acidified iso-propanol as 0.05 ml / well. Optical density of dissolved crystals was measured at 570 nm using ELISA plate reader (Biotek-ELx-800, USA). OD was plotted against concentration and the IC_{50} of GSE was determined by using *MASTER PLEX* 2010 software. [5]

Cell Cycle Analysis

MCF-7 and CaCo-2 cells were treated with the IC_{50}values of GSE and incubated at 37°C for 24 h. For cell cycle analysis the cells were harvested and fixed gently with 70% ethanol (in PBS), maintained at 4°C overnight and then re-suspended in PBS containing 40 µg/ml PI and 0.1 mg/ml RNase and 0.1% Triton X-100 in a dark room. After 30 min at 37°C, the cells were analyzed using a flow-cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an argon ion laser at 488 nm wavelength.

Expression of Apoptosis Related Genes Using Real Time PCR

Total RNA was extracted from IC_{50} GSE treated and untreated breast and colon cancer cell lines post 24 hrs treatment using RNeasy mini Kit (Qiagen - USA) according to manufacturer's instructions. Concentration of extracted RNA was evaluated using a Beckman dual spectrophotometer (USA). The expression level of apoptosis related genes; p53 (F: 5`-TCA GAT CCT AGC GTC GAG CCC-3` and R: 5`-GGG TGT GGA ATC AAC CCA CAG-3`), Bax (F: 5`-ATG GAC GGG TCC GGG GAG CA-3`and R: 5`-CCC AGT TGA AGT TGC CGT CA-3`) and Bcl-2 (F: 5`-GTG AAC TGG GGG AGG ATT GT-3`and R: 5`-GGA GAA ATC AAA CAG AGG CC-3`) was determined using real-time PCR. [7] Ten ng of the extracted total RNA from each sample were used for cDNA synthesis using High Capacity cDNA Reverse Transcriptase kit (Applied Biosystems-USA). The obtained cDNA was subsequently amplified using Syber Green I PCR Master Kit (Fermentas- Lithuania) using Step One instrument (Applied Biosystems-USA), as follows: 10 min at 95ºC for enzyme activation followed by 40 cycles of 15 secs at a temperature of 95°C, 20 sec at 55°C and 30 sec at 72°C for the amplification step. Changes in the expression of each target gene were normalized relative to the mean critical threshold (CT) values of β-actin as housekeeping gene by the ΔCt method.

ROS Measurement

DCF-DA (2', 7'-dichlorofluorescin di-acetate) and DHE (Dihydroethidium) were used to examine the effect of GSE on intracellular generation of ROS (H_2O_2 and superoxide) according to manufacturer's protocol. For DCF-DA staining, cells were cultured, washed with Krebs-ringer bicarbonate solution and incubated with 20 lM freshly prepared DCF-DA solution in dark. After 30 min, DCF-DA was removed and cells were washed with Krebs-ringer bicarbonate solution, followed by incubation with 40 lg/ml of GSE. Finally, the fluorescence was measured at different time-points using Spectramax Gemini EM fluorescence plate reader (Molecular Devices, Sunnyvale, CA) with excitation filter set at 485 nm and emission filter set at 530 nm. For DHE staining, cells were treated with GSE (40 lg/ml) followed by incubation with 10 lM DHE and analyzed for fluorescence intensity by flow cytometry. Results were obtained by gating the DHE fluorescence versus linear side scatter of cells.

Statistical Analysis

All experiments were carried out three independent times. Results were statistically analyzed using one-way analysis of variance (ANOVA) and were presented as mean ± SD. The difference was considered statistically significant at $p<0.05$.

RESULTS

Cytotoxicity

Evaluating the cytotoxic effect of GSE to human colon (CaCo-2) and breast (MCF-7) cells using MTT assay as cell viability tests indicated a dose dependent reduction in cellular viability along with increasing the concentration of GSE 24 h post treatment recording an IC_{50} values in the order of 148.5 µg/ml and 190.2 µg/ml for CaCo-2 and MCF-7 respectively. Recorded results indicated a significant greater cytotoxic potential to CaCo-2 that to MCF-7 cells ($P<0.01$) (Figure 1).

Cell Cycle Analysis

Analysis of cell cycle using flow cytometry revealed that GSE induced a statistically significant ($p<0.05$) arrest of both CaCo-2 and MCF-7 cells at G2/M phase. The percentage of arrested cells was elevated by about double fold in GSE treated cells compared to untreated control cells (Figure 2).

Expression of Apoptosis Related Genes

Evaluation of apoptotic stimulating activity was detected in both cell lines post treatment and was accompanied by a potential up regulation of pro-apoptotic (Bax) genes. Meanwhile, down regulation of the anti-apoptotic gene (Bcl-2) was recorded as well ($P<0.05$). On the other hand, there was no significant difference in the expression level of P53 gene in GSE treated cells compared to untreated cells (Figure 3).

Generation of ROS

Regarding the ROS concentration post MCF-7 and CaCo-2 cancer cells GSE treatment, it was found that ROS level was significantly elevated in the order of 40.8 % post MCF-7 treatment. On the other side, CaCo-2 cells exhibited a lower increase in the generated level of ROS (28.5 %) (Figure 4).

DISCUSSION

Natural resources have always been a better supplement than chemicals in drug manufacture and disease treatment especially that chemicals often have hazardous short or long term side effects [2] The anti-cancer potential of grape seeds was questioned for long time, however, noticeable results were recorded post treatment with procyanidine (grape seed extract). Unlike chemotherapy, grapes are

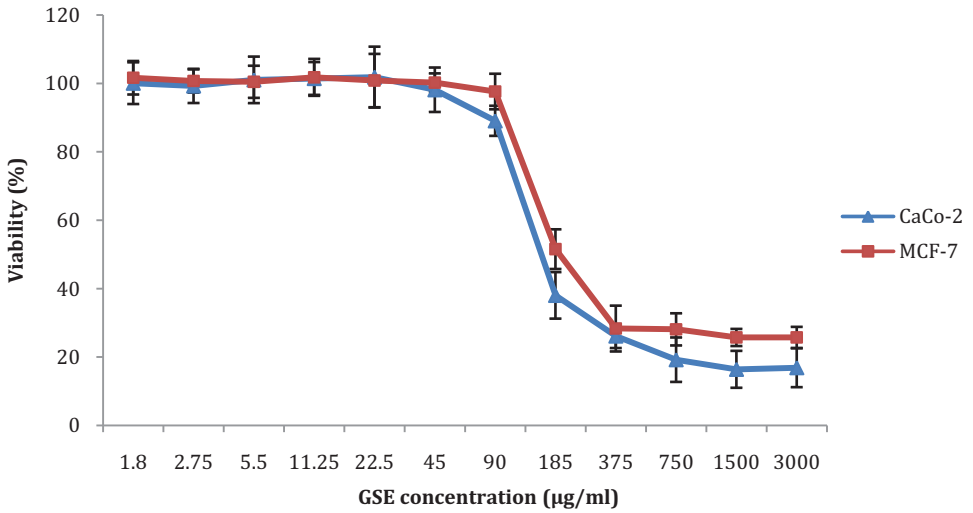

Figure 1: Evaluation of cytotoxic activity of GSE to CaCo-2 and MCF-7 cancer cells using MTT revealing dose dependent increase in cellular viability upon increasing GSE concentration. GSE exhibited slightly greater cytotoxicity to CaCo-2 than that to MCF-7 cells. Results were expressed as mean of three independent test±SD

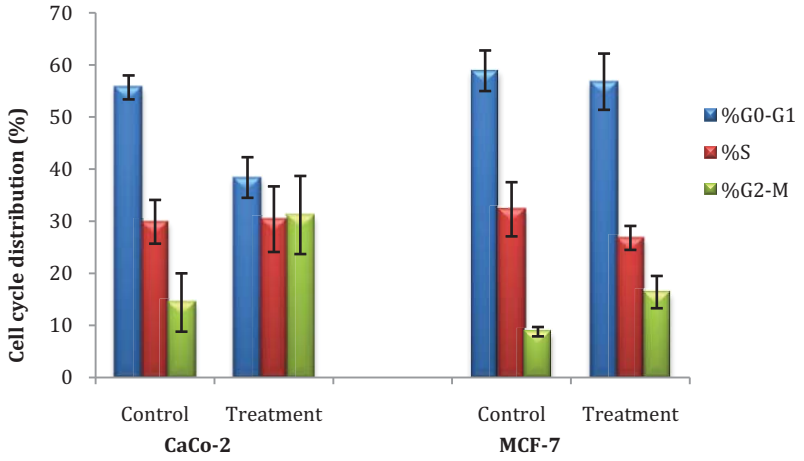

Figure 2: Analysis of cell cycle using flow cytometry post PI staining indicates an obvious G2/M phase cell cycle arrest 24 hr post exposure to IC_{50} of GSE in both CaCo-2 and MCF-7 cell lines

considered to be a natural treatment source which helps in eliminating cancerous cells without damaging other cells. In agreement with the current study, cytotoxicity assay revealed that IC_{50} of GSE on oral squamous carcinoma (KB) cells was 245.984 µg/ml post 24 hr treatment. [7] Another study demonstrated that, GSE exhibited cytotoxic potential to skin cancer (A4321) cell line with an IC_{50} value of 480 µg/ml. [8]

The ability of procyanidine to target the apoptotic pathways which will eliminate the risk of harming healthy cells through directing cancer cells to self-destruction. Previous studies showed that the grape seeds contain high amounts of polyphenols; procyanidine that regulates ROS levels. The up-regulation of pro-apoptotic genes (P53 and Bax) and down-regulation of anti-apoptotic (BCL-2) gene lead to the activation of caspase 9 and 3, however, these effects are dependable on the inhibition of PI3k/Akt pathway. GSE was shown to activate and stabilize P53 gene which reduces the levels of PI3k and phosphorylated Akt resulting in the increase of pro-apoptotic proteins leading to the enhancing of apoptosis process. Other records demonstrated that GSE might be an effective in reacting to tumors as chemotherapeutic agent through inhibition of cyclins and cyclin-dependent kinases (CDKs) and up-regulation of cyclin-dependent kinase inhibitor protein (Cdki) which induce cell cycle arrest and inhibit cell growth. [9] The antioxidants present in grape seed was found to protect DNA damage by inducing hydrogen peroxide and by adding Fe^{2+} to reduce the oxidative stress in the cells. [10] The antioxidant potential of grape is apparent in its role to act as free radicals scavenger which leads to the reduction of oxidative stress in the cells by the regulation of ROS since oxidative conditions has role in the initiation and regulation of apoptosis. There is no doubt that ROS has an important role in the cell apoptosis. [11] Data recorded pointed out that intake of grapes antioxidants significantly affected the mitochondrial enzyme system in the rat mucosal tissue resulting in protection of normal chronic mucosa from ROS attack. [12]

Grape seed extract (GSE) from Italia, Palieri and Red Globe cultivars inhibits cell growth and induces apoptosis in Caco-2 human colon cancer cells in a dose-dependent

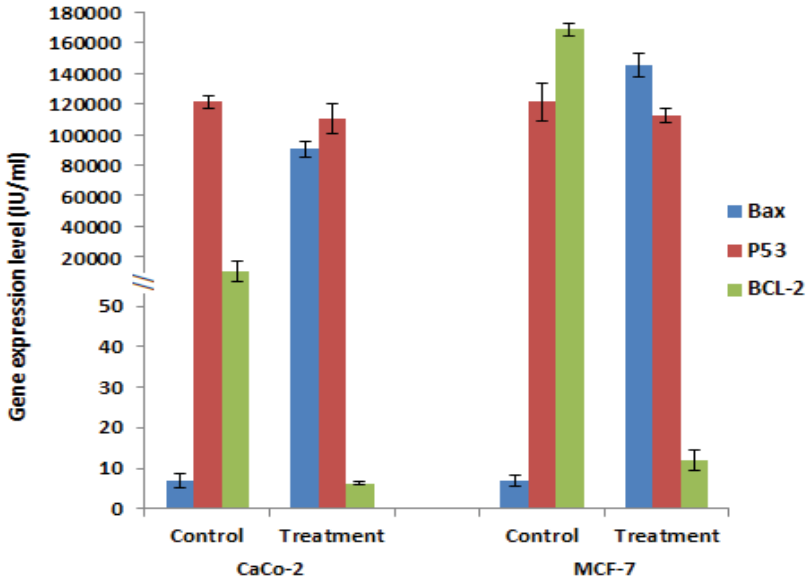

Figure 3: Assessment of expression of apoptosis related gene using real-time PCR. Recorded results demonstrated a significant up-regulation of Bax gene, while down-regulation of BCL-2 gene

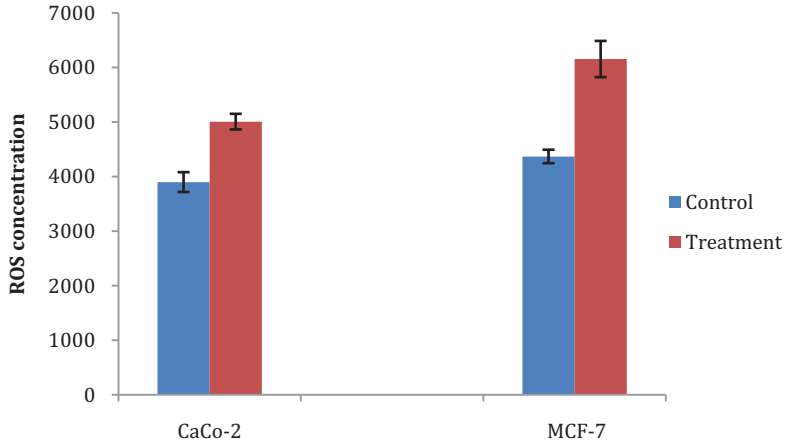

Figure 4: Estimation of the generated level of ROS post GSE treatment recorded an elevated level of ROS in MCF-7 cells greater than that recorded in CaCo-2 cells. Test was carried out in independent triplicates

manner. In order to investigate the mechanism(s) supporting the apoptotic process, they determined reactive oxygen species (ROS) production, intracellular Ca2þ handling and extracellular signal-regulated kinase (ERK) activation. Upon exposure to GSE, ROS and intracellular Ca2þ levels increased in Caco-2 cells, concomitantly with ERK inactivation. As ERK activity is thought to be essential for promoting survival pathways, inhibition of this kinase is likely to play a relevant role in GSE-mediated anticancer effects. Indeed, pretreatment with N-acetyl cysteine, a ROS scavenger, reversed GSE-induced apoptosis and promoted ERK phosphorylation. This effect was strengthened by ethylene glycol tetra acetic acid-mediated inhibition of extracellular Ca2þ influx. ROS and Ca2þ influx inhibition, in turn, increased ERK phosphorylation and hence almost entirely suppressed GSE-mediated apoptosis. These data suggested that GSE triggers a previously unrecognized ERK-based mechanism, involving both ROS production and intracellular Ca2þ increase, eventually leading to apoptosis in cancer cells. [13]

Grape is very important in the breast cancer treatment as it was shown that its antioxidants can modifies the estrogen receptor (ER) due to their similar structure to estrogen hormone. [14] Moreover, it was found that grape seed antioxidants repressed the activation of mutagen-activated protein kinase (MAPK) in breast cancer MDA-MB-468 cells and it has an essential effect on cell cycle arrest by promoting the expression of p21/ protein G1-phase arrest. Furthermore, *Vitis vinifera* can target the transcription factor, nuclear factor and kappa B by hindering its DNA-binding capability to prevent the invasion of cancerous cells. [15]

The effect of grape on breast cancer displayed hopeful outcomes. Procyanidins, which is a polyphenolic compound extracted from grape seeds, inhibits the initiation of MAPK/ERK1/2 and MAPK/p38 and results in an induction of CDKI Cip1/p21 and a reduction in CDK4 in MDA-MB-468 cells. Also, the uncontrolled growth of breast cancer, whether *in-vivo* or *in-vitro*, was shown to be in relation to constitutive activation of ERK1/2 pathway. Procyanidins in

addition resulted in cell cycle G1 arrest and subsequently the cell growth was irreversibly reduced in a significant degree. [16] In colon carcinoma (HT29) cells, cell cycle arrest was obvious in cancer cells post GSE treatment. It was explained that GSE helped in the upregulation of p21 through the activation of ERK1/2 pathway leading to the cell cycle arrest. [17]

Another study reported that the inhibition of both CaCo-2 and HCT-8 colon cancer cells growth was more effective by GSE than the isolated procyanidins, suggesting a potential anti-cancer effect by extracting more grape seed components. [18] Moreover, the addition of grape skin to the grape seed extract had a more substantial apoptotic effect on colon cancer cells by a significant upregulation of with p53 levels and Bax and a down regulation of Bcl-2. [19] Procyanidins dimer, found in grape seeds, is a highly inhibitor of the expression and consequently the activity of aromatase enzyme. In aromatase-transfected MCF-7 breast cancer cells, aromatase enzyme is essential because it plays a role in the conversion of androgens into estrogens. [20]

GSE selectively inhibited the growth and caused cell cycle arrest and apoptotic death in both Detroit 562 and FaDucancer cells by activating DNA damage checkpoint cascade as well as caspases 8, 9 and 3 in addition to accumulation of intracellular reactive oxygen species. In the meantime, GSE feeding to nude mice decreased Detroit 562 and FaDuxeno graft tumor growth by 67 and 65%, respectively. [21] Regarding oral cancer cells, it was reported that the use of different concentration of GSE on the cancer cells showed various expressions of oxidative stress and DNA damage which eventually leads to apoptosis. [22] Another explanation for the GSE anticancer potentials were justified by its relation with Ca^{2+} signaling pathways. It is commonly known that Ca^{2+} exhibits an inhibition of cell growth and promotion of cell differentiation activities in various malignant epithelial cells. [13]

In the current study, cell cycle analysis and gene expression pattern of apoptosis genes demonstrated that the absence of regulatory effect on the expression level of p53 indicated that the anti-cancer potential of GSE occurs in a p53-independent manner which is potentially related to the recorded G2/M phase cell cycle arrest. It was also suggested that the intrinsic pathway may be the initiating pathway in GSE-mediated apoptotic cell death. These findings highlights the importance of GSE in multi targeted cancer therapy especially in cancer cells exhibiting mutation in P53 gene and are subsequently resistant to treatment. [22]

CONCLUSION

From the released data it can be concluded that the mechanism of anticancer potential of procyanidine based on the absence of regulatory effect on the expression level of p53 indicated that the anti-cancer potential of GSE occurs in a p53-independent manner which is potentially related to the recorded G2/M phase cell cycle arrest. Also, GS extracted procyanidine can eliminate the risk of harming healthy cells through directing cancer cells to self-destruction and triggering previously unrecognized ERK-based mechanism, involving both ROS production and intracellular Ca2þ increase, eventually leading to apoptosis in cancer cells.

RECOMMENDATIONS

Finally, it can be recommended that the application of procyanidine can be used in different formulae with different transporting systems to be targeted. Also, its huge effect on a wide range of cancer cell lines and *in-vivo* application must be considered. Different mechanisms should be traced to clarify the actual mechanism of action of grape seed compared to the current used anticancer drugs, in order to explain the pathological changes detected.

REFERENCES

1. Siegel R L, Miller K D, Jemal A. Cancer statistics. CA Cancer J Clin, 66(1):7–30, 2016.
2. Partridge A H, Burstein H J, Winer E P. Side effects of chemotherapy and combined chemohormonal therapy in women with early-stage breast cancer. Journal of the National Cancer Institute, 93(30):135-42, 2001.
3. Zhou K, Raffoul J J. Potential anticancer properties of grape antioxidants. Journal of oncology, 1-8, 2012.
4. Loebinger M R, Kyrtatos P G, Turmaine M Price A N, Pankhurst Q, Lythgoe M F, Janes S M. Magnetic resonance imaging of mesenchymal stem cells homing to pulmonary metastases using biocompatible magnetic nanoparticles. Cancer Research, 69(23):8862–8867, 2009.
5. Shebl R I, Mohamed A F, Ali A E, Amin M A. *Cerastes cerastes* and *Vipera lebetina* snake venoms apoptotic – stimulating activity to human breast cancer cells and related gene modulation. J Cancer Sci Ther, 4:317-323, 2012.
6. Aghbali A, Hosseini S V, Delazar A, Gharavi N K, Shahneh F Z, Orangi M, Bandehagh A, Baradaran B. Induction of apoptosis by grape seed extract (*Vitis vinifera*) in oral squamous cell carcinoma. Bosn J Basic Med Sci, 13(3):186-91, 2013.
7. Mohan srinivasan V, Devi C, Deori M, Biswas A, Naine S. Exploring the anticancer activity of grape seed extract on skin cancer cell Lines A431. Brazilian Archives of Biology and Technology, 58(4):540-546, 2015.
8. Dinicola S, Cucina A, Antonacci D, Bizzarri M. Anticancer effects of grape seed extract on human cancers: A Review. Journal of Carcinogenesis and Mutagenesis, S8(005):1-14, 2014.
9. Vinson J A, Proch J, Bose P. Mega Natural® gold grape seed extract: *in-vitro* antioxidant and *in-vivo* human supplementation studies. Journal of Medicinal Food, 4(1):17-26, 2001.
10. Matés J M, Sanchez-Jimenez F M. Role of reactive oxygen species in apoptosis: implications for cancer therapy. The International Journal of Biochemistry and Cell Biology, 32(2):157-170, 2000.
11. Lopez-Oliva M E, Agis-Torres A, Goni I, Munoz-Martinez E. Grape antioxidant dietary fibre reduced apoptosis and induced a pro-reducing shift in the glutathione redox state of the rat proximal colonic mucosa. British Journal of Nutrition, 103(08):1110-1117, 2010.
12. Dinicola, Simona, Maria Addolorata Mariggio, Caterina Morabito, Simone Guarnieri, Alessandra Cucina, Alessia Pasqualato, Fabrizio D Anselmi, Sara Proietti, Pierpaolo Coluccia and Mariano Bizzarri. 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, 110(5):797-809, 2013.
13. Klinge C M, Risinger K E, Watts M B, Beck V, Eder R, Jungbauer A. Estrogenic activity in white and red wine extracts. Journal of Agricultural and Food Chemistry, 51(7):1850-1857, 2003.
14. Singh T, Sharma S D, Katiyar S K. Grape proanthocyanidins induce apoptosis by loss of mitochondrial membrane potential of human non-small cell lung cancer cells *in-vitro* and *in- vivo*. PloS One, 6(11):27444, 2011.
15. Agarwal C, Sharma Y, Zhao J, Agarwal R. A polyphenolic fraction from grape seeds causes irreversible growth inhibition of breast carcinoma MDA-MB468 cells by inhibiting mitogen-activated protein kinases activation and inducing G1 arrest and differentiation. Clinical Cancer Research, 6(7):2921-2930, 2000.
16. Kaur M, Tyagi A, Singh R P, Sclafani R A, Agarwal R, Agarwal C, Grape seed extract upregulates p21 (Cip1) through redox-mediated activation of ERK1/2 and posttranscriptional regulation leading to cell cycle arrest in colon carcinoma HT29 cells. Molecular Carcinogenesis, 50(7):553-562, 2011.
17. Dinicola S, Cucina A, Pasqualato A, D Anselmi F, Proietti S, Lisi E, Pasqua G, Antonacci D, Bizzarri M. Antiproliferative and apoptotic effects triggered by grape seed extract (GSE) versus epigallocatechin and procyanidins on colon cancer cell lines. International Journal of Molecular Sciences, 13(1):651-664, 2012.
18. Radhakrishnan S, Reddivari L, Sclafani R, Das U N, Vanamala J. Resveratrol potentiates grape seed extract induced human colon cancer cell apoptosis. Front Biosci Elite Ed, 3:1509-1523, 2011.
19. Eng E T, Ye J, Williams D, Phung S, Moore, R E, Young M K, Gruntmanis U, Braunstein G, Chen S, Suppression of estrogen biosynthesis by procyanidin dimers in red wine and grape seeds. Cancer Research, 63(23):8516-8522, 2003.
20. Sangeeta S, Gagan D, Mallikarjuna G, Manjinder Kaur, Anil K J, Swetha I, Rajesh A, Chapla A. Generation of reactive oxygen species by grape seed extract causes irreparable DNA damage leading to G2/M arrest and apoptosis selectively in head and neck squamous cell carcinoma cells. Carcinogenesis, 33(4):848–858, 2012.
21. Yen C Y, Hou M F, Yang, Z W, Tang J Y, Li K T, Huang H W, Huang Y H, Lee S Y, Fu T F, Hsieh C Y, Chen B H. Concentration effects of grape seed extracts in anti-oral cancer cells involving differential apoptosis, oxidative stress and DNA damage. BMC Complementary and Alternative Medicine, 15(1):1, 2015.
22. Derry M, Raina K, Agarwal R, Agarwal C. Differential effects of grape seed extract against human colorectal cancer cell lines: The intricate role of death receptors and mitochondria. Cancer Letters, 334(1):69-78, 2013.

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