WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 9, 291-315. Conference Article ISSN 2277– 7105

ANTICANCER EFFECTS OF GRAPE SEED EXTRACT ON HUMAN CANCER: A REVIEW

Purnima Baghel* and Anish Chandy

School of Pharmacy, Chouksey Engineering College Bilaspur (C.G.), India.

Article Received on
19 March 2018,
Revised on 09 April 2018,
Accepted on 29 April 2018
DOI: 10.20959/wjpr20189-12109

***Corresponding Author**
Purnima Baghel
School of Pharmacy,
Chouksey Engineering
College Bilaspur (C.G.),
India.

ABSTRACT

Grape seed extract (GSE) is a complex mixture of several compounds, mostly represented by polyphenols and phenolic acids. Their consumption is safe and is recognized to exert several and meaningful health benefits. In particular, grape-related anti-tumoral activity encompasses a wide array of biological mechanisms and cellular targets, eventually leading to inhibition of cell growth and to enhanced apoptosis in several cancer cell lines, including lung, colon, breast, bladder, leukemia and prostate tumors. Those effects are likely modulated at the molecular level through selectively modulating the redox balance and displaying anti-oxidant as well as pro-oxidant actions, according to the specific context. GSE-related anti-cancer activity mostly relies on the induced increase in reactive oxygen species, followed by the orchestrated down- and up-regulation of several key-molecular pathways, including MAPK kinases, PI3K/Akt, NF-kB, cytoskeleton proteins and metalloproteinases. This review will focus on Grape seed extract (GSE) may have a great relevance as source of potential new pharmacological molecules, and could represent an important opportunity for clinical research in the context of cancer.

KEYWORDS: Grape seed extract (GSE), Apoptosis, Chemoprevention, and Oxidation.

INTRODUCTION

Grape seeds and fruits

Cancer is among the leading cause of death in the Western world and its incidence is rising sharply in the developing countries too. By no doubt, that trend can be likely ascribed to the world-wide adoption of western dietary habits, characterized by high saturated fat diet, low intake of fresh vegetables and fruits, with reduced assumption of polyphenolic-rich foods

(like green tea, soy and grape seeds).[1] On the contrary, high and regular consumption of polyphenolic-rich foods has proven to significantly reduce the incidence of breast, lung, prostate and gastro-intestinal human cancers.[2] Among those foods, a prominent role is undoubtedly sustained by grapes and grape-related aliment and beverages. From time immemorial grapes have been used both for medicinal and nourishment purposes, chiefly in Greece and in Italy. Grapes (*Vitisvinifera*) have been heralded for their medicinal and nutritional value for thousands of years: Egyptians ate grapes at least 6,000 years ago, and several ancient Greek philosophers praised the healing power of grapes, usually in the form of wine. The role that the grape has in the food culture of the Mediterranean countries is comparable only to that played by tea in among the peoples of Asia, indeed. An impressive body of the current scientific literature supports the health benefits claimed by the medical tradition.

Several epidemiological studies have associated the consumption of grapes, wine, and grape juice with a wide variety of health-promoting effects, particularly the reduced risk of cancer and cardiovascular diseases.[3-6] It is worth of mentioning that a significant linear decrease in risk of lung cancer associated with consumption of red wine among eversmokers has been recorded by a multiethnic cohort study involving more than 80,000 men: consumption of 1-2 cup of wine reduces the risk of lung cancer of approximately 60%. A similar trend has been observed by other studies.[7-10] Interestingly, a similar pattern has been recorded by epidemiological studies performed on Green Tea.[11-14] Tea and grape have different chemical composition.[15] Yet, many GSE components (epigallo-catechins, procyanidins, flavonoids) are also found in Green Tea, and they may well account for the widely recognized beneficial effects of tea consumption. However, even if a consistent overlap has been observed in between the biological properties of both mixtures, yet extracts from grapes and tea differ significantly in their effectiveness, given that when they are simultaneously added to cancer cells, a synergistic, significant effect can be observed.[16] Yet, the beneficial properties of both tea and grape (or grape derived food products), are believed to be related to their polyphenolic content[17,18]; and, by no doubt, grapes constitute one of the major sources of phenolic compounds among fruits.[19]

GRAPE SEED COMPOSITION

Grape seed composition differs significantly in between different cultivars[20-23], namely when white versus red grapes are considered. Yet, those differences reflect not only genetic

variability, but also highlight the impact of vineyard treatments, ripeness grade[24,25], irrigation strategy[26,27] and nitrogen fertilization.[28] Even within seeds obtained from the same cultivar a significant variability in chemical composition has been recorded, and such a result may be likely ascribed to differences in the extraction method.[29-31] In addition, several environmental and biological factors, such as hyperopic, light, drought, high salinity, cold, metal ions, pollutants, xenobiotics, toxins, reoxygenation after anoxia, experimental manipulations, pathogenic infection and ageing of plants may affect yields and seed quality, mainly by inducing oxidative stress.[32,33] Nonetheless, plant cells have a wide array of detoxifying enzymes and pharmacologically active, anti-oxidant compounds that scavenge Reactive Oxygen Species (ROS), participate in seed survival, and may hence display relevant pharmacological activities.[34] Besides some minor components, main grape seed constituents are represented by polyphenols, phenolic and hydroxy-benzoic acids. Stylbenes (trans-resveratrol) as well, are occasionally found, even if in a few varieties.[35]

Polyphenols (Flavonoids) is a collective noun given to several classes of structurally similar compounds, having a common C6-C3-C6 flavone skeleton in which the three-carbon bridge between the phenyl groups is commonly cyclized with oxygen. Flavonoids include several classes of compounds: Flavones (luteolin), Flavan-3-ols (catechins, epicatechins, epigallo-catechins, epigallocatechin-3-gallate, procyanidins), Flavanones (neringein), and Flavonols (quercetin, rutin, kaempherol) and Anthocyanins.[36] Each class differs from the other according to the degree of unsaturation and oxidation of the three-carbon segment.[37] Flavonoids are usually present in nature as glycosides: the sugar moiety attached to the flavonoid structure affects ease of absorption from the intestinal tract and the bioavailability of the compound. Yet, glycosylation lessens the reactivity of flavonoids against free radicals and slow-down their intestinal absorption[38] Grape seeds have higher content of both phenolic acids and flavonoids (where they account for 60-70% of dry extract) [39] than grape skin and whole grape extract, meanwhile resveratrol and anthocyanidins are more abundant in the latter two extracts.[40] Several individual grape seed components have been demonstrated to display relevant chemical and biological functions, such as antioxidant[41], anti-inflammatory[42], inhibition of platelet aggregation[43], antimicrobial[44], and “anti-aging” activities.[45] Those properties have been found to be directly associated to the total polyphenolic content[46,47] and specifically ascribed to the activity of the more effective components, among which ellagic[48] and gallic acid[49], epigallocatechin-3-gallate[50], procyanidins[51] and quercetin[52] are by far the most important. Gallic acid, procyanidins and

epigallo-catechins overall account for about 80-90% of dry extract[53], and medical properties of grape seeds are generally referred to those molecules, indeed.[54]

Yet, the contribution of other active, even less represented molecules cannot be excluded, given that some biological functions seem to be synergistically afforded by interactions among the different components.[55] Namely, the well-known anti-oxidant effects exerted by GSE, can only barely be explained by the sum of the anti-oxidant activities of each individual component. Indeed, correlation analysis showed that none of the identified polyphenols had a strong correlation with protection from ROS.[56] Thus, it seems that there may be a synergism between polyphenols and or between polyphenols and phenolic acids and other phytochemicals. Similarly, even if anticancer effects are generally thought to be exerted mainly by procyanidins and epigallo-catechin-3-gallate (EGCG), again the overall GSE anticancer effect is higher than that obtained by the sum of each individual component.[57]

Chemical Structures

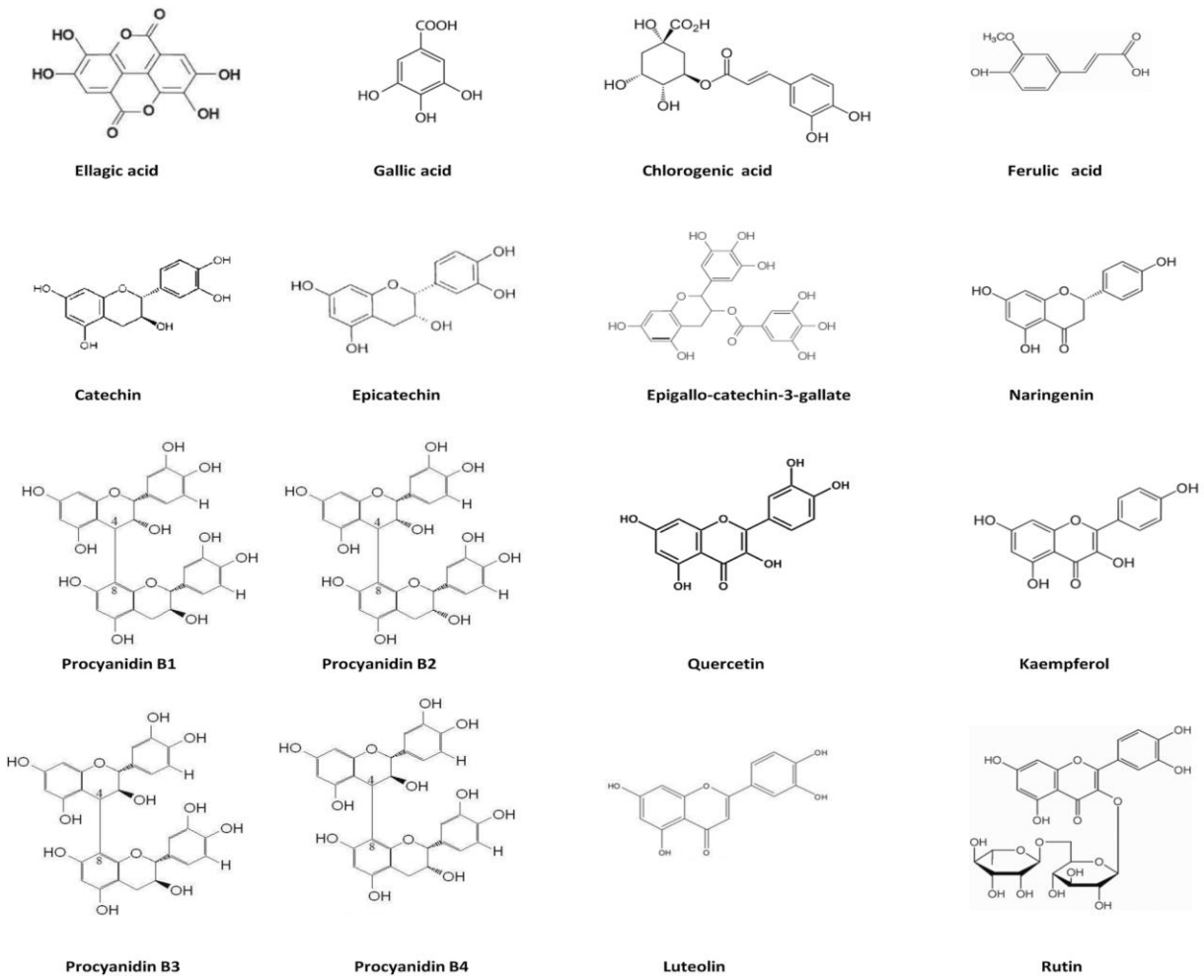

Figure 1: Chemical structures of the principal GSE components.

BIOLOGICAL ACTIVITY OF GRAPE SEED EXTRACTS

Antitumor activity

Many of the phytochemicals present in plants are generally accepted as contributors toward these health positive effects.[58] Grapes and grape-based products are one such class of dietary products that have shown cancer chemopreventive potential and are also known to improve overall human health.[59] Bomser et al examined the antitumor promoting activity of a polyphenolic fraction from grape seeds in CD-1 mouse skin epidermis. The final number of tumors per mouse in the 5, 10, and 20 mg grape seeds polyphenolic-treated animals was decreased 63, 51, and 94%, respectively, compared to controls. These studies indicate that grape seed polyphenolic extract possesses antitumor promoting activity when applied to CD-1 mouse skin.[60] Aluyen et al performed a systematic review to determine whether resveratrol is effective as an anticancer agent. The major mechanisms of actions in which resveratrol works include proapoptotic, anti-proliferation, and anti-inflammation. In conclusion, resveratrol appears to have anticancer effects.[61] In one study, Del Follo-Martinez et al investigated the anticancer activity of resveratrol and quercetin in combination (1:1 ratio) in HT-29 colon cancer cells. Moreover, gallic acid, a natural polyphenol present in a wide range of fruits and vegetables, has been of potential interest as an anticancer agent.[62] Kaur et al evaluated the efficacy of grape seed gallic acid in androgen-independent DU145 and androgen-dependent-22Rv1 human prostate cancer cells.

Gallic acid decreased cell viability in a dose-dependent manner in both DU145 and 22Rv1 cells largely via apoptosis induction.[63] Zhang et al investigated the synergistic antitumor effect of grape seed proanthocyanidin and doxorubicin both in vitro and in vivo. Approximately 100 mg/L proanthocyanidin 12.5 mg/L inhibited proliferation of K562, A549, and CNE cells in vitro in a time- and concentration-dependent manner. These results suggest that proanthocyanidin enhances the doxorubicin-induced antitumor effect and its mechanism is attributed to the promotion of doxorubicin-induced apoptosis through increasing intra-cellular doxorubicin, Ca2+ and Mg2+ concentrations, and reducing pH value and mitochondrial membrane potential.[64] In another study, Ye et al assessed the cytotoxicity of grape seed proanthocyanidin extract against MCF-7 human breast cancer cells, A-427 human lung cancer cells, CRL-1739 human gastric adenocarcinoma cells, and K562 chronic myel-ogenous leukemic cells. Concentration- and time-dependent cytotoxic effects of grape seed proanthocyanidin extract were observed on the MCF-7 breast cancer, A-427 lung cancer, and gastric adenocarcinoma cells. These data demonstrate that grape seed proanthocyanidin

extract exhibited cytotoxicity toward some cancer cells, while enhancing the growth and viability of the normal cells which were examined.[65] Zhao et al assessed the antitumor-promoting effect of a polyphenolic fraction isolated from grape seeds and employed the 7,12-dimethylbenz[a]anthracene-initiated and 12-*O*-tetradecanoylphorbol 13-acetate promoted SENCAR mouse skin two-stage carcinogenesis protocol as a model system. The observed antitumor-promoting effects of grape seed proanthocyanidin extract were dose-dependent and were evident in terms of a reduction in tumor incidence (35 and 60% inhibition), tumor multiplicity (61 and 83% inhibition), and tumor volume (67 and 87% inhibition), respectively. Procyanidin B5-3′-gallate showed the most potent antioxidant activity with an IC_{50} of 20 microM in an epidermal lipid peroxidation assay. The results show that grape seed polyphenols possess high antitumor-promoting activity due to the strong antioxidant effect of procyanidins present therein. In summary, grape seed polyphenols in general, and procyanidin B5-3′-gallate in particular, should be studied in more detail to be developed as cancer chemopreventive and/or anti-carcinogenic agents.[66] Recently, Tyagi et al identified procyanidin B2 3,3″-di-*O*-gallate as the most active constituent of grape seed extract for efficacy against prostate cancer. Both B2 3,3″-di-*O*-gallate preparations inhibited cell growth, decreased clonogenicity, and induced cell cycle arrest and apoptotic death, comparable to each other, in various human prostate cancer cell lines.[67]

Antioxidant activity

Grape seed extract is derived from the grape seeds that is extracted, dried, and purified to produce polyphenolic compound-rich extract that also has well documented antioxidant, antimicrobial, and anti-inflammatory properties.[68] Jayaprakasha et al evaluated the antioxidant activity of grape seed extracts using β-carotene-linoleate model system and linoleic acid peroxidation method. The results showed that different extracts had 65%–90% (scavenging rate) antioxidant activity at 100 ppm concentration. The present work indicated grape seed extracts may be exploitable for the preservation of food products as well as for health supplements and nutraceuticals.[69] Shaker evaluated the antioxidative effect of red grape seed and peel ethanolic extracts on primary and secondary lipid oxidation in sunflower and conjugated sunflower oils. After 6 days, a high antioxidative effect was found for the secondary oxidation products in conjugated sunflower for peel extract followed by seed extract.[70] In one study, Kim et al evaluated the effect of heating and physical conditions of grape seeds on the antioxidant activity of their extracts. The results indicated that antioxidant activity of grape seed extract was affected by heating conditions and physical conditions of

grape seeds at the time of heat treatments.[71] Brannan and Mah determined the antioxidant effect of grape seed extract by assessing the bleaching of pyrogallol red by peroxynitrite or iron/ascorbate, and the formation of lipid hydroperoxides and thiobarbituric acid substances in raw or cooked ground muscle during refrigerated or frozen storage. In this model, grape seed extract was more effective than gallic acid in inhibiting oxidation. These results show that grape seed extract at concentrations as low as 0.1% is a very effective inhibitor of primary and secondary oxidation products in various muscle systems and has potential as a natural antioxidant in raw and cooked meat systems.[72] Furiga et al investigated the effect of a grape seed extract on two oral anaerobes closely associated with periodontal diseases and its antioxidant action. The evaluation of antioxidant activity was based on the capacity of a sample to scavenge the ABTS radical cation as compared to a standard antioxidant (Trolox).

It significantly decreased the formation of biofilm. High Trolox equivalent antioxidant capacity was registered and this extract exhibited greater antioxidant capacity than vitamins C and E.[73] Moreover, Sung and Lee evaluated the antioxidant and antiproliferative activities of grape seeds from ten different cultivars. The antioxidant activity of the grape seeds was determined by radical scavenging activities and reducing power. Both the polyphenol and flavonoid contents were positively correlated with radical scavenging activity (R2.0.9). These results may provide basic information about health-beneficial effects of grape seeds.[74] Jara-Palacios et al evaluated the antioxidant potential of white grape pomaces from nine different varieties. Grape pomaces exhibited different quantitative phenolic profiles and different antioxidant activities, with significant differences (P,0.05).[75] The mechanism of antitumor activity might be associated with free radical production inhibition and regulation.

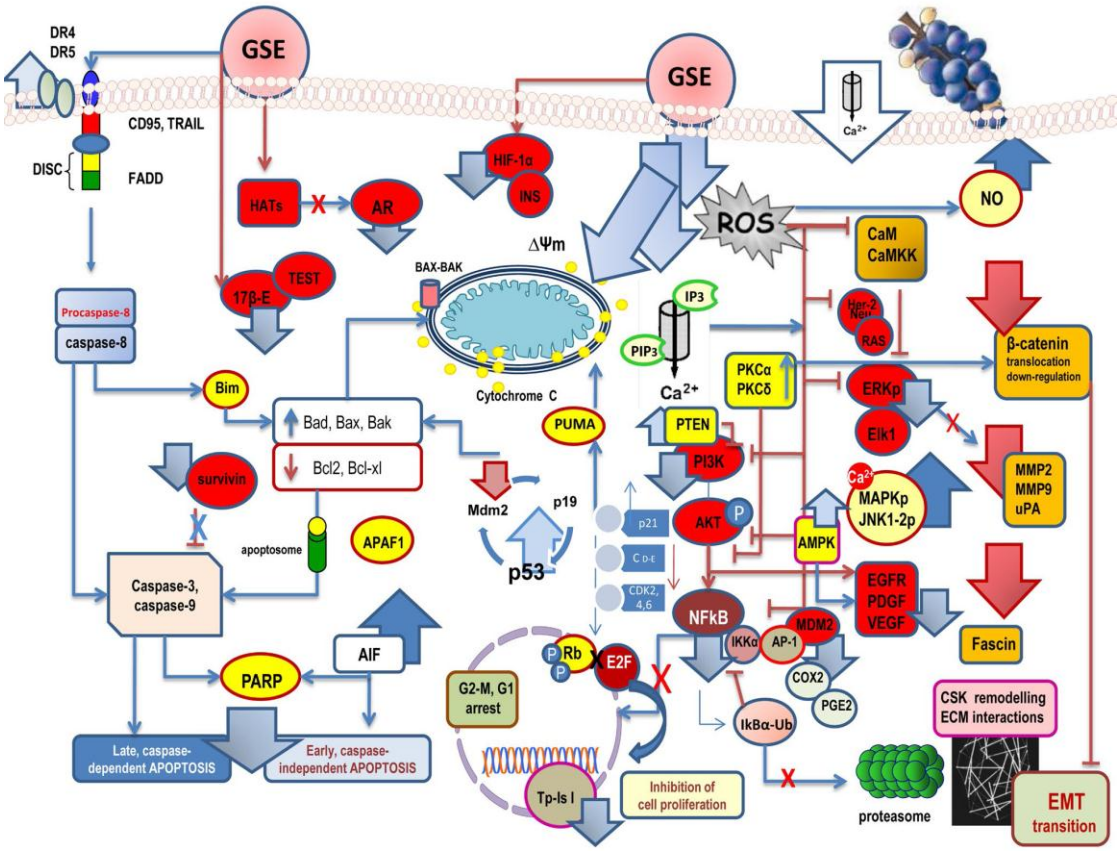

Figure 2: Molecular mechanisms of GSE interactions. GSE interacts with many cellular biochemical and genetic pathways, through which cell proliferation, cytoskeleton rearrangement, apoptosis and cell differentiation are modulated. A pivotal key-step is supported by the increase in ROS formation induced in cancer cells by GSE through the selective regulation of the redox balance. Acronymous: DISC, death-inducing signaling complex; FADD, Fas associated death domain; transmembrane death receptors, DR4, DR5; tumor necrosis factor-related apoptosis-inducing ligand (TRAIL); Insulin, INS; cAMP response elementbinding protein ,CREB); poly-ADP-ribose polymerase, PARP; FOXO1, forkhead box O1; histone androgen acetyltransferase, HATs; Apoptosis Inducing factor, AIF; Apoptotic protease activating factor 1, APAF; Hypoxia-inducible factor 1-alpha, HIF-α; testosterone, TEST; Androgen receptor, AR; Inositol-3-phospgate, IP3; Phosphatydyl-Inositol-3-phosphate, PIP3; Calmodulin, CaM; Calmodulin kinase, CaMKK; PKC, protein kinase C; p53 upregulated modulator of apoptosis, PUMA; phosphatase and tensin homologue deleted on chromosome ten, PTEN; nuclear factor kappa B, NF-kB; inhibitor of kappa B, IK-b (comprising the subunits IKKα,β,γ; Mouse double minute 2 homolog, MDM2; activator protein 1, AP-1; Nitric oxide, NO; urokinase-type plasminogen activator, uPA; matrix metalloproteinases,MMP2-9; Topoisomerase-I, Tp-Is 1; Prostaglandin-endoperoxide

synthase 2 or cyclooxygenase-2, COX-2; prostaglandin E2, PGE2; 5' AMPactivated protein kinase, AMPK; ETS domain-containing protein, Elk-1; cyclin, C; cyclin-dependent kinases, CDK; ubiquitination complex, Ub; cytoskeleton, CSK; extra-cellular matrix, ECM; epithelial-mesenchymal transition, EMT.

Reactive Oxygen Species (Ros), Mitochondrial Potential and Calcium

GSE, as well as many grape polyphenols and phenolic acids, have been shown to induce significant inhibition of cell proliferation and to enhance apoptosis in several cancer cell lines. Those effects occur at both low and high GSE concentration, the necrotic processes becoming more evident for the highest GSE doses. Such effects have been recently demonstrated to be dependent on ROS formation, occurring early after GSE administration in lung, bladder and colon cancer cells.[76] Concomitantly to ROS enhanced formation, the mitochondrial membrane potential was significantly reduced, dose and time-dependently in GSE treated cancer Cells.[77] Similar findings have been also reported by adding tea polyphenols to a wide array of cancer cell lines.[78] Those effects were long-lasting, as the decrease in mitochondrial potential still remains after 3-6 hours.[79,80] The mitochondrial transmembrane potential is often used as an indicator of cellular viability and metabolic activity, and its disruption has been involved in a variety of apoptotic phenomena.[81]

Moreover, mitochondria have also been implicated in ROS generation during apoptosis. Indeed, reduced mitochondrial membrane potential has recently been shown to lead to increased generation of ROS and apoptosis.[82] Furthermore, mitochondria are central players in cellular Ca2+ signalling given that they contribute in shaping and buffering cellular Ca2+ signals.[83,84] It is widely recognised that Ca2+ displays growth inhibiting and differentiation-promoting activities in a variety of normal and malignant epithelial cells. We have reported[85] that intracellular Ca2+ rapidly increased after the addition of GSE to cell cultures. This effect might be due to the mobilisation of intracellular Ca2+ stores, or to the influx of extracellular Ca2+. In order to address these issues, Caco-2 colon cancer cells were incubated in a Ca2+-free medium containing the Ca2+ chelator EGTA, before addiction of GSE obtained from different grape cultivars. Addition of EGTA does not modify intracellular concentration of Ca2+ in *Red Globe*-treated cells, indicating that modification in intracellular Ca2+ was tightly dependent on extracellular Ca2+ influx in this very case. However, addition of EGTA to the medium supplemented with GSE obtained from *Italia* and *Palieri* cultivars, slightly reduced but did not completely inhibit the increase observed in Ca2+ intracellular

levels, thus demonstrating that Ca2+ release in these specific cases is largely due to the depletion of intracellular Ca2+ stores. Yet, addition EGTA abolished almost completely GES-induced apoptosis on colon cancer cells as well as mitochondrial depolarisation, thus suggesting the two phenomena are entrenched. Further addition of NAC did not modify significantly those results, suggesting that ROSinduced Ca2+ release is a mandatory step in anticancer effects triggered by GSE. As previously suggested[86], those data outline a crosstalk signalling in between Ca2+ and ROS: ROS may regulate the activity of Ca2+-activated channels and, at the same time, increased Ca2+ levels could reinforce ATP synthesis-induced ROS generation. GSE-induced elevation in intracellular calcium levels is also associated to a dramatic downregulation of Calmodulin A (CaM) in breast cancer cells.[87] CaM binds to calcium and hence activates several pathway involved in cancer progression, and increased levels of CaM have been found in cancer cells.[88] However, uncoupled Ca2+ activates the RAF/MEK/ERK pathway and promotes phosphorylation of MAPKp38 and JNK, eventually leading to over-expression of p53.[89]

PRO-APOPTOTIC EFFECTS OF GSE

GSE and MAPK kinases

The extensive investigations with the GSE have identified various molecular targets involved in GSE-mediated cancer cell apoptosis. The PI3K/Akt pathway plays a pivotal role in mammalian cell survival signaling and has been shown to be activated in various cancers.[90] Indeed, phosphorylated PI3K and Akt are thought to be key factors in modulating down-stream kinases activation and NF-kB-dependent pathways. It is worth of noting that grape and tea polyphenols[91], as well as GSE, have been shown to decrease the PI3K levels and Akt phosphorylation, even enhancing proteasome degradation of Akt in several cancer cell lines.[92] Down-regulation of the phosphorylated form of PI3K is a key event in Akt regulation: Akt binds to phosphatidylinositol- 3-phosphate (PIP3), and PI3K induces its phosphorylation at the carboxy-terminal of Ser473 residue. PI3K is negatively regulated by the phosphorylated form of phosphatase and tensin homologue deleted on chromosome ten (PTEN), a lipid phosphatase that catalyzes the dephosphorylation of PIP3 and thus inhibit PI3K/Akt phosphorylation.[93] Absence of PTEN strongly correlates with activation of PI3K/Akt in tumour cell lines[94], whereas GSE significantly decreased PTEN phosphorylation, and thereby increased its negative regulation on the PI3-K pathway.[95] Phosphorylated Akt may in turn activate survival pathways by directly phosphorylating specific targets. Indeed, Akt negatively regulates factors that promote the expression of death

genes (Bad)[96] and positively regulates antiapoptotic factors (Bcl-2, CREB)[97,98] and pro-survival genes (FHKR, NFkB).[99,100] GSE significantly inhibited Akt-dependent FKHR phosphorylation in Caco-2 cells, thus leading to FHKR proteins residing predominantly in the nucleus where they are able to promote transcription of pro-apoptotic target genes such as Fas-L and Bim through specific DNA elements in their promoters. In addition, GSE suppresses Akt-related effects on CREB, NFkB[101], BAD and Bcl-2, thus promoting an overall pro-apoptotic effect on cancer cells. MAPKs signaling pathway is an important upstream regulator of transcriptional factor activities and their signaling affects a wide variety of extracellular stimuli into intracellular events and thus control the activities of downstream transcription factors implicated in cancer development and progression.[102] GSE has been reported by many studies to enhance the activation of JNK and p38MAPK, through a pathway requiring intracellular calcium increase.[103]

In turn, p38MAPK enhances apoptosis through Bcl-2 inactivation, caspase increase and mitochondria depolarization.[104] That effect has been related to ROS[105] and intracellular calcium increase and it is generally thought to participate in enhancing the overall GSE-induced apoptotic action on cancer cells. Yet, opposite findings have been recorded in normal cells.[106] Moreover, GSE and several different polyphenols from both grape and tea have been showed to exert contradictory effects on ERK1/2 activation: meanwhile some studies reported epigallocatechin-3-gallate phosphorylation of ERK1/2[107], we and others have observed a selective inhibition of ERK phosphorylation in colon and prostate cancer cells treated with GSE[108,109,110], or even EGCG.[111,112] Indeed, both down- and up-regulation of ERK activation in cancer cells have been reported occurring after treatment with GSE or isolated polyphenols.[113] Those contradictory results may be ascribed to differences in the cell culture, to dose-dependent dual effects, or to the prevalence of a specific single bioactive component, given that opposite effects on ERK activation have been documented by using different single bioflavonoids.[114] Therefore, data provided by experimental models need to be interpreted according to a systemic approach, i.e. by taking into consideration the dynamic interplay of several other observables.[115] In some way GSE and many dietary pholyphenols seem also to modulate the complex array of PKC iso-enzymes, leading to increased PKCα activation.[116] GSE may activate PKC, namely the PKCα and PCKδ isoforms, probably by increasing intracellular Calcium[117], and promoting PCKδ translocation into the nucleus, where PKC act as proapoptotic factor.[118] PKCα, together with PCKδ, could participate in inhibiting Akt phosphorylation and in triggering the extrinsic apoptotic cascade, especially in

prostate cancer cells.[119] However, the interplay in between GSE and PKC dynamics is very poorly understood and deserves further investigation. Several studies have indicated that elevated levels of inflammation modulators are functionally related to tumor promotion. Prostaglandins are produced in abundance by the metabolic conversion of arachidonic acid by COX-2, which has been known to be upregulated in a number of malignancies. Four transcription factors including nuclear factor kappa B (NF-kB), CCAAT/enhancer-binding protein (C/EBP), activator protein 1 (AP-1) and CRE-binding protein (CREB) have been identified to bind to the cis-acting elements required to promote COX-2 expression.[120]

Among the aforementioned factors, NF-kB and AP1 play a relevant role in cancer development and progression.[121] The NF-kB proteins can be activated by a wide variety of stimuli that relieve NF-kB from the inhibition exerted by IkBα. NF-kB is indeed constrained in the cytosol by binding to IkBα. NF-kB activation requires necessarily that this association be disrupted. Almost all activators of NF-kB do so by phosphorylating IkBα when bound to NF-kB–Ikα kinases resulting in accelerated degradation NF-kB and nuclear translocation of free NF-kB.[122] In the nucleus, NFkB targets different gene promoters, enhancing pro-survival pathways and even COX-2 genes expression. *In vitro* treatment of human epidermoid carcinoma A431 cells with GSE down-regulates the constitutive expression or basal level of NF-κB/p65 and IKKα and simultaneously inhibits the degradation of IκBα protein.[123] Indeed, many polyphenols as well as GSE have been proven to down-regulate NF-kB[124,125-127], and COX-2 expression. As for EGCG extracted from tea[128], NFkB down-regulation by GSE may also involve inhibition of Her-2/neu receptor tyrosine phosphorylation, an oncogene member of the EGFR family thought to play a relevant role during cancer development. To our best knowledge, among dietary flavonoids, only EGCG[129], Flavones[130,131] and mangiferin[132] (an apple procyanidin), share with GSE that meaningful, inhibitory property on NFkB activation. Eventually, GSE has been reported to down-regulate the activator protein-1 (AP-1) levels in cancer cells[133], likely through different, synergistic biochemical pathways, as it was demonstrated by using isolated polyphenols.[134] AP-1 is very often portrayed as a general, nuclear decision maker that determines the final fate of the cell upon stimulation by extracellular signals, and its down-regulation has been claimed to participate in inhibiting antiapoptotic and pro-survival pathways.[135] Additionally GSE and tea polyphenols have been demonstrated to modulate androgen[136] as well as estrogen signalling[137,138], involving a plethora of growth factor, as EFG/EGFR[139], PDGF[140], VEGF[141] and IGFBP-3.[142] Overall, these effects may converge

towards the aforementioned pathways, enhancing the anticancer activity displayed by GSE on cancer cells.

CONCLUSION AND FUTURE PROSPECTIVE

Completed studies from various scientific groups conclude that both grapes and grape-based products are excellent sources of various anticancer agents and their regular consumption should thus be beneficial to the general population. Further studies are needed, however, with individual phenolic compounds of grape seeds to elucidate the different antioxidant mechanisms and possible synergism. Moreover, further research involving electrostatic spray and nanoscale delivery of the active components present in these grape seed extracts and using them as a component in multiple hurdle approach would enhance the food safety and quality in addition to providing alternative "green" solutions to the food processors.

REFERENCES

1. Key TJ, Allen NE, Spencer EA, Travis RC The effect of diet on risk of cancer. Lancet, 2002; 360: 861-868.
2. Glade MJ Food, nutrition, and the prevention of cancer: a global perspective. American Institute for Cancer Research/World Cancer Research Fund, American Institute for Cancer Research, 1997. Nutrition, 1999; 15: 523-526.
3. Vidavalur R, Otani H, Singal PK, Maulik N. Significance of wine and resveratrol in cardiovascular disease: French paradox revisited. Exp Clin Cardiol, 2006; 11: 217-225.
4. Pezzuto JM Grapes and human health: a perspective. J Agric Food Chem., 2008; 56: 6777 6784.
5. Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med., 1995; 155: 381-386.
6. Yang CS, Landau JM, Huang MT, Newmark HL Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr, 2001; 21: 381- 406.
7. German JB Nutritional studies of flavonoids in wine: Flavonoids in Health and Disease: Rice-Evans CA, Packer L (Eds), Marcel Dekker, New York1997.
8. Ruano-Ravina A, Figueiras A, Barros-Dios JM Type of wine and risk of lung cancer: a case-control study in Spain. Thorax, 2004; 59: 981-985.
9. Chao C. Associations between beer, wine, and liquor consumption and lung cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev, 2007; 16: 2436-2447.

10. De Stefani E, Correa P, Deneo-Pellegrini H, Boffetta P, Gutiérrez LP, et al. Alcohol intake and risk of adenocarcinoma of the lung. A case-control study in Uruguay. Lung Cancer, 2002; 38: 9-14.
11. Yang CS. Inhibition of carcinogenesis by tea. Nature, 1997; 389: 134-135.
12. Sun CL, Yuan JM, Koh WP, Yu MC Green tea, black tea and breast cancer risk: a meta-analysis of epidemiological studies. Carcinogenesis, 2006; 27: 1310-1315.
13. Suzuki Y, Tsubono Y, Nakaya N, Suzuki Y, Koizumi Y, et al. Green tea and the risk of breast cancer: pooled analysis of two prospective studies in Japan. Br J Cancer, 2004; 90: 1361-1363.
14. Kuroda Y, Hara Y. Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat Res., 1999; 436: 69-97.
15. Crupi P, Coletta A, Anna Milella R, Perniola R, Gasparro M, et al. HPLCDAD- ESI-MS analysis of flavonoid compounds in 5 seedless table grapes grown in Apulian Region. J Food Sci., 2012; 77: C174-181.
16. Morré DM, Morré DJ Anticancer activity of grape and grape skin extracts alone and combined with green tea infusions. Cancer Lett., 2006; 238: 202-209.
17. Frederiksen H, Mortensen A, Schrøder M, Frandsen H, Bysted A, et al. Effects of red grape skin and seed extract supplementation on atherosclerosis in Watanabe heritable hyperlipidemic rabbits. Mol Nutr Food Res., 2007; 51: 564-571.
18. Zern TL, Wood RJ, Greene C, West KL, Liu Y, et al. Grape polyphenols exert a cardioprotective effect in pre- and postmenopausal women by lowering plasma lipids and reducing oxidative stress. J Nutr,. 2005; 135: 1911-1917
19. Alonso Borbalán AM, Zorro L, Guillén DA, Barroso CG. Study of the polyphenol content of red and white grape varieties by liquid chromatographymass spectrometry and its relationship to antioxidant power. J Chromatogr A, 2003; 1012: 31-38.
20. Castillo-Muñoz N, Gómez-Alonso S, García-Romero E, Hermosín-Gutiérrez I. Flavonol profiles of Vitis vinifera red grapes and their single-cultivar wines. J Agric Food Chem., 2007; 55: 992-1002.
21. Cavaliere C, Foglia P, Marini F, Samperi R, Antonacci D.et al. The interactive effects of irrigation, nitrogen fertilisation rate, delayed harvest and storage on the polyphenol content in red grape (Vitis vinifera) berries: A factorial experimental design. Food Chem., 2010; 122: 1176-1184.
22. Santos-Buelga C, Francia-Aricha EM, Escribano-Bailòn MT Comparative flavan-3-ol composition of seeds from different grape varieties. Food Chem., 1995; 53: 197-201.

23. Cantos E, Espín JC, Tomás-Barberán FA Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MSMS. J Agric Food Chem., 2002; 50: 5691-5696.
24. Kennedy JA, Matthews MA, Waterhouse AL Effect of maturity and vine water status on grape skin and wine flavonoids. Am J Enol Vitic, 2002; 53: 268-274.
25. Kennedy JA, Matthews MA, Waterhouse AL Changes in grape seed polyphenols during fruit ripening. Phytochemistry, 2000; 55: 77-85.
26. Esteban MA; Villanueva MJ; Lissarrague JR Effect of irrigation on changes in the anthocyanin composition of the skin of cv Tempranillo (Vitis vinifera L) grape berries during ripening. J Sci Food Agric, 2001; 81: 409-420.
27. Perez-Magariño S, González-San José MA Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. Food Chem., 2006; 96: 197-208.d
28. Choné X, Lavigne-Cruège V, Tominaga T, Van Leeuwen C, Castagnède, et al. Effect of vine nitrogen status on grape aromatic potential: flavor precursors (scysteineconjugates), glutathione and phenolic content in Vitis vinifera L. cv. Sauvignon blanc grape juice. J int Sci Vigne Vin, 2006; 40: 1-6.
29. Pekic B, Kovac V, Alonso E, Revilla E Study of the extraction of proanthocyanidins from grape seeds. Food Chem., 1998; 61: 201-206.
30. Mandic AI, Dilas SM, Cetkovic GS, Canadanovic-Brunet JM, Tumbas VT Polyphenolic Composition and Antioxidant Activities of Grape Seed Extract. Int J Food Properties, 2008; 11: 713-726.
31. Yilmaz Y, Toledo RT Oxygen radical absorbance capacities of grape/ wine industry by products and effect of solvent type on extraction of grape seed Polyphenols. J Food Comp Anal, 2006; 19: 41-48.
32. Weidner S, Karamac M, Amarowicz R, Szypulska E, Golgowska A. Changes in composition of phenolic compounds and antioxidant properties of Vitis amurensis seeds germinated under osmotic stress. Acta Physiol Plant, 2007; 29: 283-290.
33. Bartosz G. Oxidative stress in plants. Acta Physiol Plant, 1997; 19: 47-64.
34. Farrant JM, Bailly C, Leymarie J, Hamman B, Côme D, et al. Wheat seedlings as a model to understand desiccation tolerance and sensitivity. Physiol Plant, 2004; 120: 563-574.
35. Cho YJ, Kim JE, Chun HS, Kim CT, Kim SS, et al. Contents of resveratrol in different parts of grapes. Korean J Food Sci and Technol, 2003; 35: 306-308.

36. Butkhup L, Chowtivannakul S, Gaensakoo R, Prathepha P, Samappito S. Study of the phenolic composition of Shiraz red grape cultivar (Vitis vinifera L.) cultivated in North-eastern Thailand and its antioxidant and antimicrobial activity. S Afr J EnoL Vitic, 2010; 31: 89-98.
37. Hughes RJ, Croley TR, Metcalfe CD, March RE A tandem mass spectrometric study of selected characteristic flavonoids. Int J Mass Spectrom, 2001; 210: 371-385.
38. Hollman PC, Katan MB Dietary flavonoids: intake, health effects and bioavailability. Food Chem Toxicol, 1999; 37: 937-942.
39. Shi J, Yu J, Pohorly JE, Kakuda Y. Polyphenolics in grape seedsbiochemistry and functionality. J Med Food., 2003; 6: 291-299.
40. 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 Compost Anal, 2008; 21: 589-598.
41. Bors W, Saran M. Radical scavenging by flavonoid antioxidants. Free Radic Res Commun, 1987; 2: 289-294.
42. Moroney MA, Alcaraz MJ, Forder RA, Carey F, Hoult JR. Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. J Pharm Pharmacol, 1988; 40: 787-792.
43. Landolfi R, Mower RL, Steiner M. Modification of platelet function and arachidonic acid metabolism by bioflavonoids. Structure-activity relations. Biochem Pharmacol, 1984; 33: 1525 1530.
44. Capasso R, Evidente A, Schivo L, Orru G, Marcialis MA, et al. Antibacterial polyphenols from olive oil mill waste waters. J Appl Bacteriol, 1995; 79: 393-398.
45. Rice-Evans C. Flavonoid antioxidants. Curr Med Chem., 2001; 8: 797-807.
46. Arnous A, Makris DP, Kefalas P. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. J Agric Food Chem., 2001; 49: 5736-5742.
47. Landbo AK; Meyer AS Ascorbic acid improves the antioxidant activity of European grape juices by improving the juices' ability to inhibit lipid peroxidation of human LDL in vitro. Int J Food Sci Technol, 2001; 36: 727-735.
48. Vattem DA, Shetty K. Biological functionality of ellagic acid: a review. J Food Biochem, 2005; 29: 234-266.
49. Veluri R, Singh RP, Liu Z, Thompson JA, Agarwal R, et al. 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: 1445-1453.

50. Pan MH, Chiou YS, Wang YJ, Ho CT, Lin JK (2011) Multistage carcinogenesis process as molecular targets in cancer chemoprevention by epicatechin-3- gallate. Food Funct, 2006; 2: 101-110.
51. Agarwal C, Veluri R, Kaur M, Chou SC, Thompson JA, et al. Fractionation of high molecular weight tannins in grape seed extract and identification of procyanidin B2-3,3'-di-O-gallate as a major active constituent causing growth inhibition and apoptotic death of DU145 human prostate carcinoma cells. Carcinogenesis, 2007; 28: 1478-1484.
52. ElAttar TM, Virji AS Modulating effect of resveratrol and quercetin on oral cancer cell growth and proliferation. Anticancer Drugs, 1999; 10: 187-193.
53. Bakkalbas E, Yemis O, Aslanova D, Artik N. Major flavan-3-ol composition and antioxidant activity of seeds from different grape cultivars grown in Turkey. Eur Food Res Technol, 2005; 221: 792–797.
54. Guendez R, Kallithraka S, Makris DP, Kefalas P. Determination of low molecular weight polyphenolic constituents in grape (Vitis vinifera sp.) seed extracts: Correlation with antiradical activity. Food Chemistry, 2005; 89: 1-9.
55. Pignatelli P, Pulcinelli FM, Celestini A, Lenti L, Ghiselli A, et al. The flavonoids quercetin and catechin synergistically inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. Am J Clin Nutr, 2000; 72: 1150-1155.
56. Apostolou A, Stagos D, Galitsiou E, Spyrou A, Haroutounian S, et al. Assessment of polyphenolic content, antioxidant activity, protection against ROS-induced DNA damage and anticancer activity of Vitis vinifera stem extracts. Food Chem Toxicol, 2013; 61: 60-68.
57. Dinicola S, Cucina A, Pasqualato A, D'Anselmi F, Proietti S, et al. Antiproliferative and Apoptotic Effects Triggered by Grape Seed Extract (GSE) versus Epigallocatechin and Procyanidins on Colon Cancer Cell Lines. Int J Mol Sci. 2012; 13: 651-664.
58. Lima CF, Pereira-Wilson C, Rattan SI. Curcumin induces heme oxygenase-1 in normal human skin fibroblasts through redox signaling: Relevance for anti-aging intervention. *Mol Nutr Food Res*., 2011; 55(3): 430–442.
59. Kaur M, Agarwal C, Agarwal R. Anticancer and cancer chemopreventive potential of grape seed extract and other grape-based products. *J Nutr.,* 2009; 139(9): 1806S–1812S.

60. Bomser JA, Singletary KW, Wallig MA, Smith MA. Inhibition of TPA-induced tumor promotion in CD-1 mouse epidermis by a polyphenolic fraction from grape seeds. *Cancer Lett*., 1999; 135(2): 151–157.
61. Aluyen JK, Ton QN, Tran T, et al. Resveratrol: Potential as anticancer agent. *J Diet*., 2012; 9(1): 45–56.
62. Del Follo-Martinez A, Banerjee N, Li X, Safe S, Mertens-Talcott S. Resveratrol and quercetin in combination have anticancer activity in colon cancer cells and repress oncogenic microRNA-27a. *Nutr Cancer*, 2013; 65(3): 494–504.
63. Kaur M, Velmurugan B, Rajamanickam S, Agarwal R, Agarwal C. Gallic acid, an active constituent of grape seed extract, exhibits anti-proliferative, pro-apoptotic and anti-tumorigenic effects against prostate carcinoma xenograft growth in nude mice. *Pharm Res*, 2009; 26(9): 2133–2140.
64. Zhang XY, Bai DC, Wu YJ, Li WG, Liu NF. Proanthocyanidin from grape seeds enhances anti-tumor effect of doxorubicin both in vitro and in vivo. *Pharmazie*, 2005; 60(7): 533–538.
65. Ye X, Krohn RL, Liu W, et al. The cytotoxic effects of a novel IH636 grape seed proanthocyanidin extract on cultured human cancer cells. *Mol Cell Biochem*, 1999; 196(1–2): 99–108.
66. Zhao J, Wang J, Chen Y, et al. Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3′-gallate as the most effective antioxidant constituent. *Carcinogenesis*, 1999; 20(9): 1737–1745.
67. Tyagi A, Raina K, Shrestha SP, et al. Procyanidin B2 3,3’’-di-O-gallate, a biologically active constituent of grape seed extract, induces apoptosis in human prostate cancer cells via targeting NF-κB, Stat3, and AP1 transcription factors. *Nutr Cancer*, 2014; 66(4): 736–746.
68. Perumalla AV, Hettiarachchy NS. Green tea and grape seed extracts – potential applications in food safety and quality. *Food Res Int*, 2011; 44(4): 827–839.
69. Jayaprakasha GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chem.*, 2001; 73: 285–290.
70. Shaker ES. Antioxidative effect of extracts from red grape seed and peel on lipid oxidation in oils of sunflower. *LWT – Food Science and Technology*, 2006; 39(8): 883–892.

71. Kim SY, Jeong SM, Park WP, et al. Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. *Food Chem.*, 2006; 97: 472–479.
72. Brannan RG, Mah E. Grape seed extract inhibits lipid oxidation in muscle from different species during refrigerated and frozen storage and oxidation catalyzed by peroxynitrite and iron/ascorbate in a pyrogallol red model system. *Meat Sci.*, 2007; 77(4): 540–546.
73. Furiga A, Lonvaud-Funel A, Badet C. *In vitro* study of antioxidant capacity and antibacterial activity on oral anaerobes of a grape seed extract. *Food Chem.*, 2009; 113(4): 1037–1040.
74. Sung J, Lee J. Antioxidant and antiproliferative activities of grape seeds from different cultivars. *Food Science and Biotechnology,* 2010; 19(2): 321–326.
75. Jara-Palacios MJ, Hernanz D, Escudero-Gilete ML, et al. Antioxidant potential of white grape pomaces: Phenolic composition and antioxidant capacity measured by spectrophotometric and cyclic voltammetry methods. *Food Research International,* 2014; 66: 150–157.
76. Dinicola S, Mariggiò MA, Morabito C, Guarnieri S, Cucina A, et al. Grape seed extract triggers apoptosis in Caco-2 human colon cancer cells through reactive oxygen species and calcium increase: extracellular signalregulated kinase involvement. Br J Nutr, 2013; 110: 797-809.
77. Kaur M, Tyagi A, Singh RP, Sclafani RA, Agarwal R, et al. 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. Mol Carcinog, 2011; 50: 553-562.
78. Hsu CP, Lin YH, Chou CC, Zhou SP, Hsu YC, et al. Mechanisms of grape seed procyanidin-induced apoptosis in colorectal carcinoma cells. Anticancer Res., 2009; 29: 283-289.
79. Hsuuw YD, Chan WH Epigallocatechin gallate dose-dependently induces apoptosis or necrosis in human MCF-7 cells. Ann N Y Acad Sci., 2007; 1095: 428-440.
80. Kaur M, Singh RP, Gu M, Agarwal R, Agarwal C. Grape seed extract inhibits *in vitro* and *in vivo* growth of human colorectal carcinoma cells. Clin Cancer Res., 2006; 12: 6194-6202.
81. Meeran SM, Katiyar SK Grape seed proanthocyanidins promote apoptosis in human epidermoid carcinoma A431 cells through alterations in Cdki-Cdk-cyclin cascade, and caspase-3 activation via loss of mitochondrial membrane potential. Exp Dermatol, 2007; 16: 405-415.

82. Marchetti P, Castedo M, Susin SA, Zamzami N, Hirsch T, et al. Mitochondrial permeability transition is a central coordinating event of apoptosis. J Exp Med., 1996; 184: 1155-1160.
83. Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, et al. Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. J Exp Med., 1995; 182: 367-377.
84. Eager KR, Roden LD, Dulhunty AF Actions of sulfhydryl reagents on single ryanodine receptor Ca(2+)-release channels from sheep myocardium. Am J Physiol, 1997; 272: C1908 1918.
85. Boitier E, Rea R, Duchen MR Mitochondria exert a negative feedback on the propagation of intracellular Ca2+ waves in rat cortical astrocytes. J Cell Biol., 1999; 145: 795-808.
86. Feissner RF, Skalska J, Gaum WE, Sheu SS Crosstalk signaling between mitochondrial Ca2+ and ROS. Front Biosci (Landmark Ed), 2009; 14: 1197-1218.
87. Hakimuddin F, Paliyath G, Meckling K. Selective cytotoxicity of a red grape wine flavonoid fraction against MCF-7 cells. Breast Cancer Res Treat, 2004; 85: 65-79.
88. Veigl ML, Vanaman TC, Sedwick WD Calcium and calmodulin in cell growth and transformation. Biochim Biophys Acta, 1984; 738: 21-48.
89. Li DW, Liu JP, Mao YW, Xiang H, Wang J, et al. Calcium-activated RAF/MEK/ERK signaling pathway mediates p53-dependent apoptosis and is abrogated by alpha B-crystallin through inhibition of RAS activation. Mol Biol Cell., 2005; 16: 4437-4453.
90. Chang F, Lee JT, Navolonic PM, Steelman LS, Shelton JG, et al. Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. Leukemia, 2003; 17: 590-603.
91. Qin J, Xie LP, Zheng XY, Wang YB, Bai Y, 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. Biochem Biophys Res Commun, 2007; 354: 852 857.
92. Hudson TS, Hartle DK, Hursting SD, Nunez NP, Wang TT, et al. Inhibition of prostate cancer growth by muscadine grape skin extract and resveratrol through distinct mechanisms. Cancer Res., 2007; 67: 8396-8405.
93. Leslie NR, Downes CP PTEN function: how normal cells control it and tumour cells lose it. Biochem J., 2004; 382: 1-11.

94. Wu X, Senechal K, Neshat MS, Whang YE, Sawyers CL The PTEN/ MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. Proc Natl Acad Sci U S A., 1998; 95: 15587-15591.
95. Engelbrecht AM, Mattheyse M, Ellis B, Loos B, Thomas M, et al. Proanthocyanidin from grape seeds inactivates the PI3-kinase/PKB pathway and induces apoptosis in a colon cancer cell line. Cancer Lett., 2007; 258: 144-153.
96. Tan Y, Demeter MR, Ruan H, Comb MJ. BAD Ser-155 phosphorylation regulates BAD/Bcl-XL interaction and cell survival. J Biol Chem., 2000; 275: 25865- 25869.
97. Skorski T, Bellacosa A, Nieborowska-Skorska M, Majewski M, Martinez R, et al. Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent pathway. EMBO J., 1997; 16: 6151-6161.
98. Pugazhenthi S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, et al. Akt/protein kinase B up-regulates Bcl-2 expression through cAMPresponse element-binding protein. J Biol Chem., 2000; 275: 10761-10766.
99. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell., 1999; 96: 857-868.
100. Dolcet X, Llobet D, Pallares J, Matias-Guiu X NF-kB in development and progression of human cancer. Virchows Arch, 2005; 446: 475-482.
101. Dinicola S, Pasqualato A, Cucina A, Coluccia P, Ferranti F, et al. Grape seed extract suppresses MDA-MB231 breast cancer cell migration and invasion. Eur J Nutr, 2013.
102. Wagner EF, Nebreda AR Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer, 2009; 9: 537-549.
103. Chen KC, Liu WH, Kao PH, Chang LS Calcium-stimulated mitogenactivated protein kinase activation elicits Bcl-xL downregulation and Bak upregulation in notexin-treated human neuroblastoma SK-N-SH cells. J Cell Physiol, 2010; 222: 177-186.
104. De Chiara G, Marcocci ME, Torcia M, Lucibello M, Rosini P, et al. Bcl-2 Phosphorylation by p38 MAPK: identification of target sites and biologic consequences. J Biol Chem., 2006; 281: 21353-21361.
105. Chen ZP, Schell JB, Ho CT, Chen KY Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. Cancer Lett, 1998; 129: 173-179.
106. Balasubramanian S, Efimova T, Eckert RL Green tea polyphenol stimulates a Ras, MEKK1, MEK3, and p38 cascade to increase activator protein 1 factor-dependent

involucrin gene expression in normal human keratinocytes. J Biol Chem., 2002; 277: 1828-1836.

107. Hudson TS, Hartle DK, Hursting SD, Nunez NP, Wang TT, et al. Inhibition of prostate cancer growth by muscadine grape skin extract and resveratrol through distinct mechanisms. Cancer Res., 2007; 67: 8396-8405.
108. Siddiqui IA, Adhami VM, Afaq F, Ahmad N, Mukhtar H. Modulation of phosphatidylinositol-3-kinase/protein kinase B- and mitogen-activated protein kinase-pathways by tea polyphenols in human prostate cancer cells. J Cell Biochem, 2004; 91: 232-242.
109. Briviba K, Pan L, Rechkemmer G. Red wine polyphenols inhibit the growth of colon carcinoma cells and modulate the activation pattern of mitogen-activated protein kinases. J Nutr, 2002; 132: 2814-2818.
110. Tyagi A, Agarwal R, Agarwal C. Grape seed extract inhibits EGFinduced and constitutively active mitogenic signaling but activates JNK in human prostate carcinoma DU145 cells: possible role in antiproliferation and apoptosis. Oncogene, 2003; 22: 1302-1316.
111. Eberhardt W, Huwiler A, Beck K-F, Walpen S, Pfeilschifter J. Amplification of IL-1 beta-induced matrix metalloproteinase-9 expression by superoxide in rat glomerular mesangial cells is mediated by increased activities of NF-kappa B and activating protein-1 and involves activation of the mitogen-activated protein kinase pathways. J Immunol, 2000; 165: 5788–5797.
112. Opare Kennedy D, Kojima A, Hasuma T, Yano Y, Otani S, et al. Growth inhibitory effect of green tea extract and (-)-epigallocatechin in ehrlich ascites tumor cells involves a cellular thiol-dependent activation of mitogenicactivated protein kinases. Chem Biol Interact, 2001; 134: 113-133.
113. Kaur M, Tyagi A, Singh RP, Sclafani RA, Agarwal R, et al. 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. Mol Carcinog, 2011; 50: 553-562.
114. Kong AN, Yu R, Hebbar V, Chen C, Owuor E, et al. Signal transduction events elicited by cancer prevention compounds. Mutat Res., 2001; 480-481: 231-41.
115. Bizzarri M, Palombo A, Cucina A. Theoretical aspects of Systems Biology. Prog Biophys Mol Biol., 2013; 112: 33-43.

116. Gossé F, Guyot S, Roussi S, Lobstein A, Fischer B, et al. Chemopreventive properties of apple procyanidins on human colon cancerderived metastatic SW620 cells and in a rat model of colon carcinogenesis. Carcinogenesis, 2005; 26: 1291-1295.
117. Sampson SR, Lupowitz Z, Braiman L, Zisapel N. Role of protein kinase Calpha in melatonin signal transduction. Mol Cell Endocrinol, 2006; 252: 82-87.
118. Zhang X-M, Chen J, Xia Y-G, Xu Q. Apoptosis of murine melanoma B16-BL6 cells induced by quercetin targeting mitochondria, inhibiting expression of PKC-alfa and translocating PKC-delta. Cancer Chemother Pharmacol, 2005; 55: 251-262.
119. Gonzalez-Guerrico AM, Kazanietz MG Phorbol ester-induced apoptosis in prostate cancer cells via autocrine activation of the extrinsic apoptotic cascade: a key role for protein kinase C delta. J Biol Chem., 2005; 280: 38982-38991.
120. Kundu JK, Shin YK, Surh YJ Resveratrol modulates phorbol esterinduced pro-inflammatory signal transduction pathways in mouse skin in vivo: NF-kappaB and AP-1 as prime targets. Biochem Pharmacol, 2006; 72: 1506-1515.
121. Perkins ND Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat Rev Mol Cell Biol., 2007; 8: 49-62.
122. Aggarwal BB Nuclear factor-kappaB: the enemy within. Cancer Cell., 2004; 6: 203-208.
123. Meeran SM, Katiyar SK Proanthocyanidins inhibit mitogenic and survival-signaling *in vitro* and tumor growth in vivo. Front Biosci, 2008; 13: 887-897.
124. Mantena SK, Baliga MS, Katiyar SK Grape seed proanthocyanidins induce apoptosis and inhibit metastasis of highly metastatic breast carcinoma cells. Carcinogenesis, 2006; 27: 1682-1691.
125. Holmes-McNary M, Baldwin AS Jr Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. Cancer Res., 2000; 60: 3477-3483.
126. Velmurugan B, Singh RP, Kaul N, Agarwal R, Agarwal C. Dietary feeding of grape seed extract prevents intestinal tumorigenesis in APCmin/+ mice. Neoplasia, 2010; 12: 95-102.
127. Mutoh M, Takahashi M, Fukuda K, Matsushima-Hibiya Y, Mutoh H, et al. Suppression of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells by chemopreventive agents with a resorcin-type structure. Carcinogenesis, 2000; 21: 959-963.
128. Pianetti S, Guo S, Kavanagh KT, Sonenshein GE Green tea polyphenol epigallocatechin-3 gallate inhibits Her-2/neu signaling, proliferation, and transformed phenotype of breast cancer cells. Cancer Res., 2002; 62: 652-655.

129. Ahmad N, Gupta S, Mukhtar H. Green tea polyphenol epigallocatechin- 3-gallate differentially modulates nuclear factor kappaB in cancer cells versus normal cells. Arch Biochem Biophys, 2000; 376: 338-346.
130. Kalra N, Seth K, Prasad S, Singh M, Pant AB, et al. Theaflavins induced apoptosis of LNCaP cells is mediated through induction of p53, down-regulation of NF-kappa B and mitogen-activated protein kinases pathways. Life Sci., 2007; 80: 2137-2146.
131. Wenzel U, Kuntz S, Brendel MD, Daniel H. Dietary flavone is a potent apoptosis inducer in human colon carcinoma cells. Cancer Res., 2000; 60: 3823- 3831.
132. Sarkar A, Sreenivasan Y, Ramesh G, Manna S beta-D-glucoside suppresses tumor necrosis factor-induced activation of nuclear transcription factor kappaB but potentiates apoptosis. J Biol Chem., 2004; 279: 33768-33781.
133. Gopalakrishnan A, Tony Kong AN Anticarcinogenesis by dietary phytochemicals: cytoprotection by Nrf2 in normal cells and cytotoxicity by modulation of transcription factors NF-kappa B and AP-1 in abnormal cancer cells. Food Chem Toxicol, 2008; 46: 1257-1270.
134. Chung JY, Huang C, Meng X, Dong Z, Yang CS Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-ras-transformed cells: structure-activity relationship and mechanisms involved. Cancer Res., 1999; 59: 4610-4617.
135. Eferl R, Wagner EF AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer, 2003; 3: 859-868.
136. Liao S. The medicinal action of androgens and green tea epigallocatechin gallate. Hong Kong Med J., 2001; 7: 369-374.
137. Kampa M, Theodoropoulou K, Mavromati F, Pelekanou V, Notas G, et al. Novel oligomeric proanthocyanidin derivatives interact with membrane androgen sites and induce regression of hormone-independent prostate cancer. J Pharmacol Exp Ther., 2011; 337: 24-32.
138. Kao YH, Hiipakka RA, Liao S Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology, 2000; 141: 980-987.
139. Sun Q, Prasad R, Rosenthal E, Katiyar SK Grape seed proanthocyanidins inhibit the invasiveness of human HNSCC cells by targeting EGFR and reversing the epithelial-to-mesenchymal transition. PLoS One, 2012; 7: e31093.

140. Labrecque L, Lamy S, Chapus A, Mihoubi S, Durocher Y, et al. Combined inhibition of PDGF and VEGF receptors by ellagic acid, a dietaryderived phenolic compound. Carcinogenesis, 2005; 26: 821-826.
141. Hwang JT, Ha J, Park IJ, Lee SK, Baik HW, et al. Apoptotic effect of EGCG in HT 29 colon cancer cells via AMPK signal pathway. Cancer Lett., 2007; 247: 115-121.
142. Barthomeuf C, Lamy S, Blanchette M, Boivin D, Gingras D, et al. Inhibition of sphingosine-1-phosphate- and vascular endothelial growth factor-induced endothelial cell chemotaxis by red grape skin polyphenols correlates with a decrease in early platelet-activating factor synthesis. Free Radic Biol Med., 2006; 40: 581-590.