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REVIEW

Review of the Pharmacological Effects of *Vitis vinifera* (Grape) and its Bioactive Constituents: An Update

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***Vitis vinifera* fruit (grape) contains various phenolic compounds, flavonoids and stilbenes. In recent years, active constituents found in the fruits, seeds, stems, skin and pomaces of grapes have been identified and some have been studied. In this review, we summarize the active constituents of different parts of *V. vinifera* and their pharmacological effects including skin protection, antioxidant, antibacterial, anticancer, antiinflammatory and antidiabetic activities, as well as hepatoprotective, cardioprotective and neuroprotective effects in experimental studies published after our 2009 review. Clinical and toxicity studies have also been examined. Copyright © 2016 John Wiley & Sons, Ltd.**

Keywords: Vitis vinifera; grape; resveratrol; flavonoids; antioxidant.

INTRODUCTION

Grape (*Vitis vinifera* L. Vitaceae) is cultivated worldwide (Afzalzadeh *et al*., 2015), and it is the world's largest fruit crop with an annual production of more than 67 million tons (Fontana *et al*., 2013). In recent years, several important reviews have described the phytochemical and pharmacological effects of grape and the active constituents in different parts of the fruit, including skin, seeds, pomace and stems (Flamini *et al*., 2013; Tang and Chan, 2014; Yang and Xiao, 2013; Xia *et al*., 2010; Teixeira *et al*., 2014).

Our previous review of *V. vinifera* was published in Phytotherapy Research (Nassiri-Asl and Hosseinzadeh, 2009). The current review provides an update of that work and includes more recently published research.

PHYTOCHEMICALS

The most important active constituents of grapes are phenolic compounds (Tang and Chan, 2014). In 2009, Amico *et al*. reported that the main constituents of the grape stem ethanolic extract are: two triterpenoid acids, oleanolic and betulinic acids; a stilbenoid, daucosterol; *E*-resveratrol and its dimer *E*-ε-viniferin; gallic acid as a simple phenol; catechin and gallocatechin (flavanols); four 6′-*O*-acyldaucosterols; and five 1,2-di-*O*-acyl-3-*O*-β-D-galactopyranosyl glycerols (Amico *et al*., 2009).

V. vinifera seeds were found to contain considerable quantities of gallic acid in addition to smaller amounts of *p*-coumaric, caffeic and ferulic acids. They contained greater amounts of both gallic and *p*-coumaric acids than the seeds of other fruit varieties (Weidner *et al*., 2013).

When Corinthian currants (*V. vinifera* L., var. Apyrena) were examined, five anthocyanidin-3-*O*-glucosides were identified and quantified, with malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside being the most abundant glucosides (Chiou *et al*., 2014).

The most abundant flavanols in red grape pomace extract (*V. vinifera* L. cv. Malbec) were (+)-catechin and (−)-epicatechin with malvidin-3-glucoside being the most abundant anthocyanin. Furthermore, for the first time, piceatannol, a stilbene analogue to resveratrol, was identified and quantified in grape pomace (Antoniolli *et al*., 2015). It was reported that the phenolic composition of *V. vinifera* L. fruits is highly dependent on the grape variety (de la Cerda-Carrasco *et al*., 2015). The different chemical groups of polyphenols identified in different fruit parts have been summarized in Fig. 1.

PHARMACOLOGICAL EFFECTS

Experimental studies

Antioxidant activities. The aqueous extracts of *V. vinifera* L. tendrils have the potential to enhance the antioxidant capacity of human keratinocytes (NCTC 2544). This effect is important as keratinocytes are often

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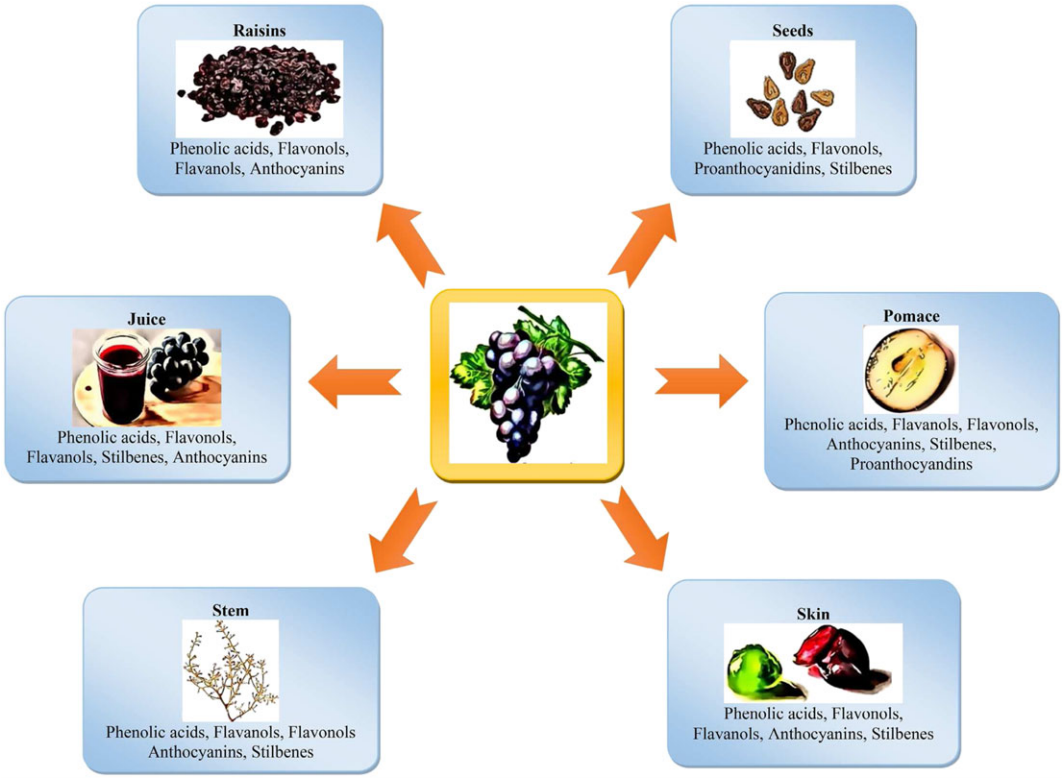

Figure 1. Different chemical groups of polyphenols identified in different fruit parts.

exposed to oxidative stress and need sufficient antioxidant defenses (Fraternale *et al.*, 2011).

Lyophilized red grape juice, at doses up to 0.01 µg, demonstrated cardioprotective effects against doxorubicin induced toxicity in cardiac-derived H9c2 myocytes. In contrast, at doses of 0.01 µg to 0.05 µg, it enhanced oxidative stress in cardiac cells, probably because of pro-oxidant effects of the juice, as indicated mainly by the increase in reactive nitrogen species and antioxidant enzyme levels (Tenore *et al.*, 2012). These findings should be considered when taking into account the recommended daily intake of polyphenols for achieving an antioxidant effect.

In addition, an inverse relationship was found between melatonin and malondialdehyde (MDA) levels in the fruit of *V. vinifera* cv. Malbec, which suggested that melatonin is an antioxidant present in grapes (Boccalandro *et al.*, 2011).

On the other hand, a polyphenol-rich extract of grape pomace has shown dual effects both *in vitro* and *in vivo*. *In vitro*, the extract scavenged free radicals and inhibited DNA damage induced by peroxyl and hydroxyl radicals, but *in vivo*, it induced oxidative stress by increasing the protein carbonyl groups in erythrocytes and heart cells, increasing plasma thiobarbituric acid reactive substances, and decreasing the concentration of glutathione in the liver (Veskoukis *et al.*, 2012). It is obvious that grape pomace extract acts differently in *in vitro* studies when compared to *in vivo* studies. If this is the case, then the findings of both *in vivo* and *in vitro* studies are not the same. A further study suggested that depending on the extraction methods or grape varieties, grape pomace extracts have antioxidant or pro-oxidant activity (Cotoras *et al.*, 2014).

In a recent study of 24 *V. vinifera* grape cultivars, it was determined that there is a direct relationship between total phenolic compounds and flavonoids and antioxidant activity (Liang *et al.*, 2014). In future studies of the antioxidant activities of grapes, researchers will be advised to investigate further the phenolic and flavonoid constituents. Some antioxidant effects of *V. vinifera* and its active constituents are summarized in Table 1.

Antibacterial, antiviral and antifungal effects. Some antiviral and anti-encephalitozoon activities of resveratrol and grapes were mentioned (Yadav *et al.*, 2009).

Resveratrol has been shown to exhibit antiviral effects against polyomavirus (Berardi *et al.*, 2009). The hot water extract of grape skin (100 mg/mL) has shown anti-influenza activity in Madine–Darby Canine Kidney (MDCK) cells (Bekhit Ael-D *et al.*, 2011). Procyanidin, an active compound of *V. vinifera* and some herbs, showed anti-influenza A activity and could inhibit the replication of this virus at several stages of life cycle (Dai *et al.*, 2012). Two dimethoxy-resveratrol derivatives (3,4′-dimethoxy-resveratrol and 3,5-dimethoxy-resveratrol) displayed interesting antifungal activities with minimum inhibitory concentration (MIC) values of 28–37 µg/mL against *Candida* species (Houillé *et al.*, 2014).

The ethanolic extract of *V. vinifera* L. tendrils has shown reasonable antifungal activities against *Fusarium* species with MIC values of 250–300 ppm. It seems that the high amounts of polyphenols in this plant play a major role in the observed antifungal effects (Fraternale *et al.*, 2015).

Preincubation of a gastric cell line with resveratrol (75 µM and 100 µM, 72 h) inhibited the secretion of IL-8 by *Helicobacter pylori*-infected cells. Pretreatment with resveratrol (1–100 µM) suppressed *H. pylori*-induced reactive oxygen species (ROS) generation. Furthermore, pretreatment with resveratrol (100 µM) blocked the *H. pylori*-induced hummingbird

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Table 1. Antioxidant effects of *V. vinifera* and its active constituents in *in vitro* and *vivo* studies

Compound	Method	Effects	References
In vivo			
Grape seed extract 100 mg/kg, p.o., 14 d before irradiation	γ-radiation-induced oxidative stress in rats	Protected heart and pancreas from oxidative damage	Saada *et al*., 2009
Burgund Mare variety, Recaş, Romania (BMR) 3 × 30 mg gallic acid equivalents/kg, p.o., on days of 1,7 and 14 of pregnancy	Pregnant rats	Increased plasma antioxidant capacity, but has no effect on NO level	Mureşan *et al*., 2010
Öküzgözü (*V. vinifera* L. cv.) grape juice, 1208 ± 43.00 μg/mL as the gallic acid equivalent and 5.2 ± 0.19 μg/mL as the quercitin equivalent, 2 mL/kg, 28 d	CCl_4-treated rats	Increased antioxidant capacity, protected against LDL oxidation and showed neuroprotective effects	Pirinççioğlu *et al*., 2012
Ethanolic extract of grape skin and flesh 2.5 g/kg. p.o, 12 w	Ethanol-induced oxidative stress in mice	Reduced oxidative stress and alteration in immune function and angiogenesis	Mukherjee *et al*., 2012
Hydroalcoholic extract of black grape 400 mg/kg, p.o., 30 d	Lead-induced oxidative stress in rats	Enhanced antioxidant capacity, increased body and organ weight and improved hematological parameters	Lakshmi *et al*., 2013
In vitro			
Polar methanol extracts of the raisins 250, 400 and 500 μg of dried product	tBHP-induced cytotoxicity in peripheral blood mononuclear cells	Showed the antioxidant and anti-atherogenic effects via scavenging free radical, reducing LDL oxidation, inhibiting total GSH decrement, cytotoxicity and morphological features of apoptosis	Kaliora *et al*., 2009
Polyphenol-rich grape pomace extract 2, 5 μg/mL, 2-h incubation	Thawed bovine sperm	Protected spermatozoa from lipid peroxidation and consequently protected sperm characteristics such as motility, viability and acrosomal integrity	Sapanidou *et al*., 2014
Condensed tannins from grape seeds Concentration 7.5–45 μg/mL	Incubation of erythrocyte with oxidant ($ONOO^-$)	Protected erythrocytes from oxidative damage	Olchowik *et al*., 2012
Phenolic extract form grape seeds Concentrations 5–100 mg/mL	H_2O_2-treated platelets	Increased the reduction of glutathione in human platelet	Kedzierska *et al*., 2011

Abbreviation: Tetrachloride carbon (CCl_4), hydrogen peroxide (H_2O_2), low density lipoprotein (LDL), Nitric oxide (NO), tert-butylhydroperoxide (tBHP).

phenomenon in cells, which is correlated with altered motility, migration and adhesion of the cells (Zaidi *et al*., 2009).

Grape extracts and their compounds were effective at inhibiting *H. pylori* in a gastric cell line, but showed differing effects against *H. pylori* growth in AGS cells. Muscadine grape skin extract demonstrated the highest efficacy (MIC range, 256–512 μg/mL) followed by muscadine seed (MIC range, 256–1,024 μg/mL) and synergy extracts (skin and seed) (MIC range, 512–1,024 μg/mL). Resveratrol and ellagic acid also inhibited *H. pylori* growth (MIC range, 6.25–50 μg/mL) (Brown *et al*., 2009). Quercetin and resveratrol, both active polyphenols in extracts of muscadine grape skin, have inhibitory effects against *H. pylori* with minimal bactericidal concentrations of 256 μg/mL and 128 μg/mL, respectively. These substances do not appear to influence the outer membrane integrity of *H. pylori*, which has previously been suggested as the mechanism for their antibacterial effect (Brown and Jiang, 2013). These results should be considered in future studies of the treatment of *H. pylori* gastrointestinal disease. Polyphenols in grape seed extract have antibacterial effects at a concentration of 3 mg/mL against methicillin-resistant *Staphylococcus aureus*, which may be by disrupting the cell wall or the cell membrane (Al-Habib *et al*., 2010). As the antibacterial, antiviral and antifungal effects of grape have been

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studied in *in vitro* studies, further studies on animals are necessary to establish these effects *in vivo*.

Anticancer effects. Since our 2009 review, several studies have focused on the anticancer effects of the extracts of different grape parts, such as stem and seed extracts. We have summarized these findings in Table 2.

Grape seed extract showed anticancer effects and induced apoptosis in colon cancer cell lines. An interesting observation is that the growth inhibition induced by Italia and Palieri grape seed extracts was significantly higher than that previously recorded with epigallocatechin and procyanidins (Dinicola *et al*., 2012). Amico *et al*. (2009) conducted the first reported bioassay on the isolated and purified constituents of the ethanolic extract of *V. vinifera* stems and evaluated their anticancer effects. Among these constituents, a triterpenoid, betulinic acid, inhibited growth by 50% (GI_{50}) in MCF-7 human breast cancer cells at a concentration of 0.57 μM (Amico *et al*., 2009). Methanol extracts of currants have cancer preventive effects and phenolic compounds may play a role in this effect (Kaliora *et al*., 2008). For more details, see Table 2.

Grape seed extract selectively induced apoptosis in different colorectal cancer cell lines (SW480, SW620 and HCT116) accompanying the release of cytochrome c in the cytoplasm of the cancer cells and the loss of mitochondrial membrane potential (Derry *et al*., 2013).

Interestingly, the grape seed extract not only enhanced the anticancer effect of 5-fluorouracil in a dose-dependent manner *in vitro*, but also reduced mucositis induced by 5-fluorouracil in rats after chemotherapy, and its protective effect was more obvious in the proximal jejunum than in the distal small intestine (Cheah *et al*., 2014a). In addition, procyanidins of mature grape seed extract increase the effectiveness of 5-fluorouracil in human colon cancer cells (Caco-2). It seems that the amount of (−)-epicatechin as a terminal subunit in the fractions of purified procyanidins in the extracts of mature seeds was greater than that in the extracts of immature seeds (Cheah *et al*., 2014b).

The anticancer effects of resveratrol metabolites, including resveratrol-3-*O*-sulfate, resveratrol-3-*O*-glucuronide and resveratrol-4-*O*-glucuronide on colon cancer cells have been established. At a concentration of 30 μM, they inhibited the proliferation of metastatic colon cancer cells and caused strong cell accumulation in the S phase of the cell cycle. At concentrations of 10 or 20 μM, they showed synergistic chemotherapeutic effects with SN38 and oxaliplatin on metastatic colon cancer cells (SW620) (Aires *et al*., 2013). These results are important as the metabolites of resveratrol could be used as a therapeutic adjunct to chemotherapeutic agents.

In parallel to the above study, resveratrol 3-*O*-D-glucuronide and resveratrol 4′-*O*-D-glucuronide (30 μM) caused G1 arrest in CCL-228 and Caco-2 cells. Resveratrol glucuronides could also reduce cyclin D_1 levels at higher concentrations (100 μM). Resveratrol 3-*O*-D-sulfate had no effect on the cell cycle in any cell line (Polycarpou *et al*., 2013).

On the other hand, the anticancer effects of resveratrol on colon cancer in both *in vivo* and *in vitro* experiments were shown to be mediated via DNA damage that was induced by producing ROS, and this response was associated with a resistance to resveratrol after prolonged treatment. An increasing dose of resveratrol could not prevent this phenomenon *in vivo*. Similarly, this resistance was observed *in vitro*. This adaptation should be considered in clinical studies that will be conducted in the future on human cancer (Colin *et al*., 2014).

Furthermore, it was reported that unlike resveratrol, its metabolites do not have any role on endothelial nitric oxide synthase (eNOS) enzyme activity, nitric oxide (NO) release or intracellular ROS production in the human endothelial cell line EA.hy92. Accordingly, there is a possibility that these metabolites are inactive (Ladurner *et al*., 2014). Although the important role of resveratrol in the treatment of cancer and inflammatory diseases has been shown in several studies, more *in vivo* studies are necessary to elucidate the mechanisms underlying effects of resveratrol and its active metabolites.

Grape seed extract has been shown to have dose-dependent protective effects in normal cells (Hfl-1) against doxorubicin toxicity. However, it increased the cytotoxicity of doxorubicin in tumor cell lines (HepG2 and Mls) (Postescu *et al*., 2012). These findings are important and illuminate the role of grape seed extract as a future supplement that is able to prevent the adverse effects of doxorubicin in chemotherapy.

Although the anticancer effects of grape have been reported in experimental studies on cell lines and animals, conducting clinical studies on grape and its active constituents is necessary to confirm this activity in human cancer. Because grape is an important constituent in our daily diet, continuous study of the role of grape and its active components in preventing cancer is recommended.

Effects on the cardiovascular system. Skin polygalloyl polyflavan-3-ols of *V. vinifera* L. are very effective inhibitors of human platelet aggregation and low-density lipoprotein oxidation *in vitro*. It was suggested that useful effects of grape are related to this compound (Shanmuganayagam *et al*., 2012).

Feeding adult male offspring of rats with a high-fat diet (24% fat) and grape skin extract (ACH09) (100 mg/kg) during lactation protected them from hypertension in later life. ACH09 could reverse increases in adiposity, plasma triglyceride levels, glucose levels, insulin resistance and oxidative stress (Resende *et al*., 2013). It seems that ACH09 may achieve this by inducing NO synthesis, exhibiting antioxidant activities and stimulating insulin signaling pathways (Resende *et al*., 2013). Thus, the administration of grape as a dietary supplement should be further investigated. More details about the cardioprotective effects of dried fruit and active constituents of *V. vinifera* are demonstrated in Table 3.

Antidiabetic effects. There are many *in vivo* studies reporting the antidiabetic activity of grape and grape byproduct polyphenols, especially grape seed extracts. Studies have shown that different proteins and enzymes are involved in these antidiabetic activities (Akaberi and Hosseinzadeh, 2016). *V. vinifera* extract (100 mg/kg and 200 mg/kg, p.o., 12 weeks) could protect prediabetic mice, in which diabetes was induced by a high-fat diet, from peripheral change measured by intraepidermal

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Table 2. A. and B. Anticancer effects of *V. vinifera* and its active components

Compound	Study	Effects	Reference
***In vitro* studies**			
V. vinifera			
Greek raisins (Dried product 500 μg/mL)	AGC cell line	Decreased protein and mRNA levels of ICAM-1 in TNF-α stimulated cells, inhibited cell proliferation by inducing apoptosis	Kaliora *et al.*, 2008
Corinthian raisins (Currants, CR) and Sultanas (S)	HT29	Showed antiproliferative, antioxidative and antiinflammatory effects	Kountouri *et al.*, 2013
Viniferin-enriched extracts 1-10 μM	MCF7, HEPG2, MRC5, HCC1500 and HCC1954 cell lines	Exhibited antiproliferative activity by modulation of the cell cycle and induction of cytotoxicity only in cancer cell lines	Giovannelli *et al.*, 2014
Stem extract (polyphenols)	MCF-7, MDA-MB-23, 786-0, Caki-1, K1, HT29 cell lines	Inhibited cell proliferation	Sahpazidou *et al.*, 2014
Stem extract (polyphenols) 345–584 mg GAE/g dry weight	HEPG2 and HeLa cell lines	Prevented DNA damage induced by ROS	Apostolou *et al.*, 2013
Seed extract 600 μg/mL	KB and HUVEC cell lines	Only induced apoptosis in KB cell line, inhibited growth of cells	Aghbali *et al.*, 2013
Seed extract 25 and 50 μg/mL 50, 100 μg/mL	Caco Caco2 and HCT-8	Inhibited growth of cells and induced apoptosis Apoptosis was induced via both caspase-dependent and caspase-independent mechanisms	Dinicola *et al.*, 2012 Dinicola *et al.*, 2012
Seed extract 40 μg/mL	Detroit 562, FaDu	Produced ROS that might be involved in inhibiting growth of cells and inducing DNA damage and apoptosis	Shrotriya *et al.*, 2012
Skin extract (Polyphenols) 5–100 μg/mL	4 T1 cells	Inhibited cell viability and migration and PI3k/Akt and MAPK pathways	Sun *et al.*, 2012
Active constituents			
Resveratrol 200 μM	HEPG2 cell line	Down-regulated cycline D1 and cell proliferation and survival pathway, inhibited p38 MAP kinase AKt/PKB and PAK1	Parekh *et al.*, 2011
50 μM	FTC-133, NPA, FRO cells	Decreased CD97 and inhibited tumor growth	Kang *et al.*, 2012
100 μM	HeLa, SiHa	Inhibited cell growth and induced apoptosis via inhibiting Wnt, Notch and STAT3 pathway	Zhang *et al.*, 2014
***In vivo* studies**			
Skin extract (Polyphenols) 0.5, 1 mg/mL drinking water	4 T1 cells was implanted s.c. in mice	Showed antitumor and inhibitory effects on lung metastasis and enhanced survival of mice	Sun *et al.*, 2012
Seed extract 0.2% in diet	Xenograft growth of Detroit 562, FaDu in nude mice	Decreased growth of xenograft tumor and increased DNA damage and apoptosis	Shrotriya *et al.*, 2012
Gallic acid 1 and 3% w/v in drinking water	DU145 and 22Rv1 xenograft growth in athymic nude mice	Inhibited growth of both cells especially 22Rv1, induced apoptosis and decreased microvessel density	Kaur *et al.*, 2009
Red grape juice 1%, 2 w before AOM	AOM-induced colon cancer	Decreased expression of COX-2 and Ki-67 that has known as important biomarker of cell proliferation	Silva *et al.*, 2015

Abbreviations: Azoxymethane (AOM), Gallic acid equivalent (GAE), Intercellular adhesion molecule 1 (ICAM-1), reactive oxygen species (ROS), tumor necrosis factor-alpha (TNF-α). **Cell lines:** Cervical cancer cell line (HeLa), colon cancer cell line (Caco2, HCT-8, HT29), gastric cancer cell line (AGC), follicular thyroid cancer cell line (FTC-133), head and neck squamous cell carcinoma (Detroit 562, FaDu), human breast cancer cell lines (HCC1500 and HCC1954), human cervical squamous carcinoma cell line (SiHa), human hepatocellular carcinoma cell line (HEPG2), human breast cancer cell line (MCF7, MDA-MB-23), human prostate cancer cells (DU145, 22Rv1), human umbilical vein endothelial cells (HUVEC), mouse mammary carcinoma cells (4 T1), normal human fibroblast cell line derived from fetal lung tissue (MRC5), Poorly differentiated papillary thyroid cancer cell line (NPA), oral squamous cell carcinoma (KB cells), renal cell lines (786-0 and Caki-1), thyroid cell line (K1), undifferentiated/anaplastic thyroid cancer cell line (FRO)

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Table 3. Cardioprotective effects of dried fruits of *V. vinifera* and its active constituents

Compound	Dose	Model	Effects	Reference
Myricetin	100, 300 mg/kg, p.o., 21 d	ISO-induced MI	Histopathological studies showed lesser degree of cellular infiltration	Tiwari *et al.*, 2009
	100, 300 mg/kg, p.o., 6 w	Fructose-induced hypertension and metabolic alteration	Prevented the development of high blood pressure and reversed metabolic alterations	Godse *et al.*, 2010
	100, 300 mg/kg, p.o., 4 w	DOCA-induced hypertension	Showed antihypertensive effects and increased the levels of antioxidant agents	Borde *et al.*, 2011
Currants	Adding 10% to normal and hypercholestrolemic diet	0.5% w/w cholesterol diet, 8 w	Reduced atherosclerotic lesion formation, decreased plasma oxidative stress and kept AST at the normal level	Yanni *et al.*, 2015

Abbreviations: Deoxycorticosterone acetate (DOCA), isoproternol (ISO), myocardial infarction (MI).

nerve fiber (IENF) length (in mm) and was independent of any effect on glucose control. Lipid profiles were also improved (Jin *et al.*, 2013).

The KCNJ11 gene encodes the kir6.2 channel. It was shown that a mutation in this gene causes congenital hyperinsulinism and has an important role in developing type 1 diabetes. Pterostilbene, as an active component of *V. vinifera*, has strong inhibitory effects on both normal and mutant kir6.2 models (Jagadeb *et al.*, 2014). Some anti-diabetic effects of *V. vinifera* and its active constituents are summarized in Table 4.

Effects on the central nervous system. Grape seed extract has prevented neuron death in H_2O_2-induced oxidative stress by upregulating IL-6 production in astrocytes (Fujishita *et al.*, 2009).

Regrapex-R, a formulation containing the combined extracts of whole grape (*V. vinifera*) and *Polygonum cuspidatum*, possesses antioxidant activity and can attenuate the loss of locomotor function in a *Drosophila* model of Parkinson's disease. One gram of Regrapex-R contains 100 mg of resveratrol complex (trans-resveratrol and its glycosides), 10 mg of emodin complex (emodin and emodin glycosides), 450 mg of polyphenols and 12 mg of anthocyanins (Long *et al.*, 2009).

Administration of resveratrol (1 g/kg, 2 months) to SAMP8 mice, a model of age-related Alzheimer's disease, improved cognition impairment, increased the life span of animals and activated adenosine monophosphate-activated protein kinase and pro-survival pathways (Porquet *et al.*, 2013).

Oligomers of resveratrol, including (+)-vitisinol, (+)-ε-viniferin, (+)-ampelopsin A, (+)-vitisin A and (−)-viticin B, that were isolated from a stembark extract of *V. vinifera*, have inhibitory effects on BACE-1 (beta-site APP-cleaving enzyme 1) *in vitro* (Choi *et al.*, 2009). BACE-1 inhibition is an important target for the treatment of Alzheimer's diseases as β-secretase in neurons is essential for the production of beta amyloid (Choi *et al.*, 2009).

Viniphenol A (1–20 μM), a recently discovered resveratrol hexamer, was isolated from *V. vinifera* and

Table 4. Antidiabetic effects of *V. vinifera* in animals

Compound	Doses	Model	Effects	Reference
V. vinifera **Seeds**	100 mg/kg, p.o., 20 d	STZ-induced diabetics in rats, 50 mg/kg, i.p.	Showed antioxidant effects against produced ROS and protected liver cells	Chis *et al.*, 2009
V. vinifera (*Muscat Variety*) **Seeds** Ethanolic extract	250, 500 mg/kg, p.o., 28d	STZ-induced diabetics in rats, 55 mg/kg, i.p.	Decreased, ALT AST and TBARS and prevent histopathological changes in liver Prevented the decrease in ICDH, SDH, MDH and G-6-PDH and increased LDH	Giribabu *et al.*, 2015
Skin Aqueous extract(ACH09)	200 mg/kg/d/p.o.	Alloxan-induced diabetics mice, 300 mg/kg, i.p.	Increased GLUT-4, glucose did not change secretion of insulin	Soares de Moura *et al.*, 2012
Delphinidine and cyanidine* chloride with and without liposome	100 mg/kg/24 h	Incubation of albumin and glucose with concentration of 100 mg/mL, 8-w STZ-induced diabetic mice, 50 mg/kg, i.v.	Both *in vivo* an *in vitro* studies, reduced rate of albumin and HbA1c glycation	Gharib *et al.*, 2013

Abbreviations: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose transporter (GLUT-4), glucose-6-phosphate (G-6-PD) lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH), succinic dehydrogenase (SDH), streptozotocin (STZ), thiobarbituric acid reactive substances (TBARS).

*Delphinidine and cyaniding: phenolic compound of grape.

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Table 5. Hepatoprotective effects of *V. vinifera* and its active components in *in vivo* and *ex vivo* studies

Study	Method	Mechanism	Reference
Red grape seeds Rat liver	Paracetamol-induced hepatotoxicity	Protected liver by decreasing the activities of liver enzymes, kidney parameters and LPO, increased the levels of GSH, SOD and CAT to the normal levels in liver	Madi Almajwal and Farouk Elsadek, 2015
Seed extract Rat liver	DEN-induced oxidative stress and hepatocellular damage	Significantly reversed elevated levels of serum SGOPT, SGOT, ALP and GGT levels, decreased LPO and enhanced serum SOD, CAT and GPx	Kumar and Vijayalakshmi, 2015
Antistax®, a standardized red vine leaf aqueous extract AS195 Rat liver and kidney	CCl4-induced toxicity	Biochemical parameters and histological evidences confirmed this hepatorenal protection	Ahmed *et al.*, 2015
Different grape cultivar extracts, seeds, skin and pulp Goat liver tissue (*ex vivo*)	Fenton-like reagent (200-μM H_2O_2, 2 mM ascorbate, 25-μM FeSO4)-induced liver damage H_2O_2-induced oxidative stress	The antioxidant levels of GSH and GSH and SOD activities were higher in seed, than in skin and pulp, the skin of Flame seedless (black) cultivar has highest antioxidant potential Skin of grape showed the stronger protective activity against oxidative stress than its pulp of any cultivar, the Flame seedless (black) cultivar has potential antioxidant activity	Singha and Das, 2014
Grape seed procyanidin extract	Fructose induced hypertriglyceridemia	Reduced hepatic lipid droplet accumulation and cholesterol content, thus, could decrease steatosis induced by fructose Decreased synthesis of TG and increased total fecal lipid excretion	Downing *et al.*, 2015

Abbreviations: Aalkaline phosphatase (ALP), carbon tetrachloride (CCl4), catalase (CAT), diethylnitrosamine (DEN), gammaglutamyl transferase (GGT), glutathione (GSH), glutathione peroxidase (GPx), lipid peroxidation (LPO), serum glutamate pyruvate transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), superoxide dismutase (SOD), triglyceride (TG).

was shown to have protective effects against amyloid-β-induced (Aβ 25–35) toxicity in PC12 cell cultures (Papastamoulis *et al.*, 2014).

Both conventional and organic purple grape juices (10 μL/g, 17 d) protected the brains of rats from lipid peroxidative damage, increased enzymatic and non-enzymatic antioxidant agents, and decreased nitric oxide in pentylenetetrazole-induced seizures. However, the protective effect of the organic juice was more than that of the conventional juice, which may be related to its higher content of polyphenols (Rodrigues *et al.*, 2012). Similar effects were reported for both organic and conventional extracts of grapevine leaves (*V. labrusca* var. Bordo) (5 mg/mL) against H_2O_2 damage (Dani *et al.*, 2010). The administration of *V. vinifera* extract (400 mg/kg, p.o., 45 d) could protect the brain from aluminum neurotoxicity (100 mg/kg, p.o., 45 d). Behavioral, histopathological and biochemical studies confirm this neuroprotective effect (Lakshmi *et al.*, 2014).

The grape seed oil of *V. vinifera* (0.87 g/mL) has neuroprotective effects against oxidative stress induced by γ-radiation and CCl_4 in rats. It decreased MDA and NO levels, increased antioxidant levels and down-regulated xanthine oxidase, iNOS and pro-inflammatory cytokines such as TNF-α, IL-6 and TGF-β_1 (Ismail *et al.*, 2015). Administration of grape seed proanthocyanidins (500 mg/kg) could improve abnormal peripheral nerve functions and impaired nervous tissues in the spinal cord of rats with type 2 diabetes mellitus. In addition, this dose showed an inhibitory effect on Ca^{2+} overload in sciatic nerves (Ding *et al.*, 2014).

Effects on inflammatory diseases. Vitisin A, a resveratrol tetramer isolated from *V. vinifera* roots, could suppress lipopolysaccharide-induced NO production via inhibiting the ERK1/2 pathway, p38 phosphorylation and NF-kB activation (Mi Jeong *et al.*, 2009).

A water extract from red vine leaves (*V. vinifera*) had inhibitory effects on TNFα-induced IL-8 secretion and expression via impairment of the NF-kB pathway in human gastric epithelial cells; this could be useful in attenuating gastric inflammation. It is suggested that quercetin glycosides are more responsible than anthocyanins for this antiinflammatory effect and that this effect is less apparent after gastric digestion. However, its antiinflammatory effect was lost after intestinal digestion; this may be related to its degradation and loss of its biological activity in the gut (Sangiovanni *et al.*, 2015).

Hepatoprotective effects. This effect was discussed in our previous review. Since then, some studies combined *V. vinifera* with other herbal medicines and investigated the effect their combination in different hepatotoxic models. It seems that the antioxidant, free radical scavenging and antiinflammatory activities of grape and other herbs are responsible for their hepatoprotective effects (Kang *et al.*, 2012; El-Beshbishy *et al.*, 2010). In one study, a diet that included 15% grape seed powder protected several tissues, including the liver, against oxidative stress induced by 20% ethanol in rats (Dogan and Celik, 2012). In this study, it was suggested that the intake of functional food is useful in the prevention of chronic degenerative liver diseases.

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Table 6. Clinical studies of *V. vinifera*

Groups	Type of study	Dose	Effects	Reference
Healthy men (42.5 ± 5.6 y) *N* = 8	Intervention	Nagano Purple grape 200-g skin or 50-g dry fruits = 1-g polyphenols blood sampling, 1 h after administration grape	Increased resistance of LDL to oxidation, It seems that this antioxidant activity is related to cyaniding-3-glucoside that has been found in Nagano grape	Kamiyama *et al.*, 2009
Elite men athlete *N* = 20, Placebo *N* = 20, Grape extract	Randomized, double-blind, placebo controlled, and crossover study on 20 elite sportsmen	Placebo (maltodextrin):400 mg/d/1 month GE: 400 mg/d/1 month Then, 2 w of wash out period, the treatments were reversed	Protected cells against oxidative stress damage via decreasing in CPK concentration and the increase of Hb level in plasma and enhanced physical Performance in handball	Lafay *et al.*, 2009
Men and postmenopausal women with T2DM Control = 27 CR = 27	Two-armed randomized controlled study	36 g/d Total polyphenol 54.5–88.5 mg equivalent to gallic acid, 24 w	Significantly decreased DBP and increased antioxidant potentials in plasma of patients	Kanellos *et al.*, 2014
Control = 4 Oil group = 15	Double-blinded study	500-mg aspirin, 7 d 1 g/d seed oil of *V. vinifera* (mostly linoleic acid), 7 d	Platelet aggregation significantly decreased in oil group compared to the control	Bazán-Salinas *et al.*, 2015
Middle-aged women: Placebo, *n* = 31 Low dose group, *n* = 33 High dose group, *n* = 32	Randomized, double-blind, placebo-controlled study	4 tablet/d of GSPE, 8 w Placebo Low dose (50 mg/d) High dose (100 mg/d)	Improved physiological and psychological symptom of menopause, increased muscle mass and decreased blood pressure	Terauchi *et al.*, 2014
Postmenopausal women: *n* = 46 4 groups	Randomized, double-blind, dose-finding early-phase trial	Grape seed extract 200, 400, 600 or 800 mg, single dose, 12 w	The levels of estrogen did not decrease, or increase androgen precursors	Wahner-Roedler *et al.*, 2014
Patients with resectable colorectal cancer *n* = 20	Intervention	Foods and drinks contain resveratrol (0.5 and 1 g/d), for 8 days prior to surgery	Significantly reduced tumor cell proliferation and level of resveratrol was higher in the cecum compared to the sigmoid colon and rectum.	Patel *et al.*, 2010

Abbreviations: Corinthian raisins (CR), creatine phosphokinase (CPK), diastolic blood pressure (DBP), grape seed proanthocyanidin extract (GSPE), hemoglobin (Hb), low density lipoprotein (LDL), type 2 diabetes mellitus (T2DM).

Some hepatoprotective effects of grapes against different hepatotoxic models *in vivo* and *ex vivo* are summarized in Table 5.

Dermatological studies. Polyphenols of red grape seed (*V. vinifera* L, var. Burgund Mare) (BM) extracts have protective effects against multiple doses of UV-B irradiation (240 mJ/cm^2, 10 d) and both dosages (2.4 and 4 mg polyphenols/cm^2) showed enhanced antioxidant activity against UV-B irradiation and inhibited apoptosis in the skin of SKH-1 mice (Filip *et al.*, 2011b). In parallel to the above study, a pretreatment combination of hydroethanolic extracts of BM with *Calluna vulgaris* (4 mg/40 μL/cm^2) had protective effects against a single dose of UV-B irradiation (240 mJ/cm^2) and decreased IL-6 and TNF-α levels compared to UV-B group in the same mouse species. It seems that the grape extract works like a sunscreen and could modulate inflammatory and apoptotic responses to UV-B irradiation in mice (Filip *et al.*, 2011a).

Furthermore, topical grape seed extract 2% w/w in a eucerin base had significant wound healing effects in rabbits and this effect was greater than that of topical phenytoin (Hemmati *et al.*, 2011).

Further study is required regarding the protective effects of grape varieties on the skin of animals and humans.

Effects on hormone replacement therapy. Grape seed extract (10 mg/kg/day, p.o., 60 d) in ovariectomized (OVX) adult female rats improved cognitive functions and prevented plasma lipid peroxidation. There were no changes in body and uterus weights. Thus, it was suggested that grape seed extract could play a useful role in the treatment of menopause symptoms (Sevastre *et al.*, 2014).

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In spontaneous hypertensive rats (SHRs), which were OVX as a model of menopause, grape seed extract was administered daily (300 mg/kg, p.o., 6 d). In OVX SHRs, urine levels of unconjugated flavonoids (catechin, epicatechin and their metabolites) were decreased compared to that in sham-OVX SHRs (Cutts *et al*., 2013). However, there were no differences in the urinary levels of total methylated or sulfated catechin between OVX and sham-OVX SHRs. It seems that unconjugated levels of catechin, epicatechin and their methylated metabolites in postmenopausal women would be less than their levels in premenopausal women after ingesting the same dose of grape seed extract (Cutts *et al*., 2013).

Effects on the reproductive system. Administration of grape juice (2 g/kg, p.o.) during the sexual maturation period of male rats until the end of one spermatogenic cycle in adult life could protect testicular morphology against cadmium toxicity, known for inducing male infertility (Lamas *et al*., 2015).

CLINICAL STUDIES

Effects on skin disorders. *V. vinifera* extract was considered as one of the best botanical ingredients for anti-aging creams in 2010 (Cronin and Draelos, 2010). One gram of trans-resveratrol (0.1% w/v), alone or with β-cyclodextrein (β-CD), was added into water-in-oil (W/O) cream (10 g) and used in a single-blinded study on eight women (ages 45–70) in each group. The creams were used twice a day, and all patients were examined on days 1 and 30. All patients had a visible improvement of clinical conditions on both hemi-faces. It seems that resveratrol was effective in the treatment of aging. Moreover, the use of resveratrol in combination with β-CD was more effective than resveratrol alone. However, the molecular mechanisms underlying this combined action are unclear (Moyano-Mendez *et al*., 2014).

Furthermore, a single-blinded randomized study was carried out on young adult healthy men ($n = 110$). In this study, application of topical water-in-oil (W/O) cream of 2% grape seed extract, or placebo, on cheek skin, twice a day for 8 weeks, has shown that the antioxidant rich formulation of grape extract has significant effects on skin melanin levels, elasticity and sebum content when compared to the placebo (Sharif *et al*., 2015).

In clinical studies, a topical formulation of *V. vinifera* A. s-I-M.t-O.dij (Ixoderm®) has shown protection against the destructive effects of radiotherapy on skin in patients with breast cancer. This study recommends hydration of the skin with a moisturizing agent such as grape as this could protect skin from the cutaneous damage caused by radiotherapy (Ravo *et al*., 2011). Some clinical effects of grape and its active constituents are summarized in Table 6.

Toxicity. Resveratrol was administered in four doses of 0.5, 1, 2.5, and 5 g to 40 healthy volunteers for 29 days to investigate its safety and pharmacokinetic properties, and those of its major metabolites. Results have shown that resveratrol at doses of 0.5 and 1 g was completely safe and that adverse gastrointestinal effects appeared with doses of 2.5 and 5 g. Maximal plasma levels (C_{Max}) and areas under the curve (AUC) of the metabolites were greater than those of resveratrol (Brown *et al*., 2010). It seems that the metabolites of resveratrol are mostly responsible for its biological effects. Resveratrol decreases circulating levels of insulin-like growth factor-1 and insulin-like growth factor binding protein-3, and this may be related to the chemopreventive effects of resveratrol (Brown *et al*., 2010).

CONCLUSION

In this review, we have summarized several pharmacological effects of *V. vinifera* extracts and their active constituents including antioxidant, anticancer, antibacterial and antidiabetic activities as well as cardioprotective, hepatoprotective and neuroprotective effects.

In recent years, it seems that the pharmacological effects of not only the whole fruit of *V. vinifera* have been studied, but studies have also been undertaken on the seeds, skin, stems and pomace as well. Human studies to establish the antioxidant, antidiabetic and antibacterial activities or cardioprotective, hepatoprotective and neuroprotective effects of grape are necessary. Equally, the protective role of grapes on the skin and the endocrine system should not be ignored. It should be noted that the extraction methods and grape varieties greatly influence the biological activity of grape extracts and future research will need to consider this. Further toxicological studies are required to determine the therapeutic doses of different grape products as this will affect clinical studies.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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