Food Research International 77 (2015) 333–348

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Blessings in disguise: Bio-functional benefits of grape seed extracts

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ARTICLE INFO

Article history:
Received 7 April 2015
Received in revised form 1 August 2015
Accepted 17 August 2015
Available online 29 August 2015

Keywords:
Grape seed extract
Polyphenol
Proanthocyanidin
By-product
Food industry
Bioactivity

ABSTRACT

Grapes (*Vitis vinifera*) are one of the largest fruit crops in the world, with an annual production of 69 million metric tons. They are a good source of polyphenolic compounds and 60–70% of these polyphenols, especially phenolic acids, ellagitannins, flavonols, flavan-3-ols such as catechins and their isomers, anthocyanins, proanthocyanidins and the stilbene derivative resveratrol exist in grape seeds. Grape seeds comprise 5% by-mass of grapes and are the major industrial by-products from grape processing industries such as grape juice and wine industries. Nowadays, grape seed extracts (GSEs) are receiving increased interest from scientists, nutritionists and consumers as a result of their reported potential health benefits for a variety of disorders. They are widely being consumed as a dietary supplement on the basis of their potent anti-oxidant, anti-cancer, antimicrobial, anti-aging, anti-hepatotoxic and anti-inflammatory activities and also have generally recognized as safe status approved by Food and Drug Administration. The beneficial activities of the grape seeds give them the possibility to be used in pharmaceutical and food industries, for medical treatments and health supplements. The review summarizes current knowledge on the bioactivities of grape seeds and provides tools for those intending to conduct research in this field as large quantities of grape seeds are discarded as waste from the wine making industry. Therefore, this by-product resulting from the grape industry could be utilized through results of diverse studies on their bioactivities.

Contents

Abbreviations: GSE, Grape seed extract; GSEs, Grape seed extracts; GSPS, Grape seed polyphenols; GSPE, Grape seed proanthocyanidin extract; GSP, Grape seed proanthocyanidin; GSPs, Grape seed proanthocyanidins; DP, Degree of polymerization; SW, Subcritical water; PA, Proanthocyanidin; PAs, Proanthocyanidins; LDL, Low density lipoprotein; GSH, Glutathione; ROS, Reactive oxygen species; MDA, Malondialdehyde; STZ, Streptozotocin; HF, High fructose; AGEs, Advanced glycation end products; PCA, Prostate carcinoma; NF-κB, Nuclear factor kappa B; CRC, Colo-rectal cancer; NO, Nitric oxide; TG, Triglyceride; AST, Aminotransferase; ALT, Alanine aminotransferase; LDH, Lactate dehydrogenase.

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http://dx.doi.org/10.1016/j.foodres.2015.08.026
0963-9969/

1. Introduction

Grapes are taxonomically classified under the order of Ramnales, the family of Vitaceae and the genus of Vitis (Vine, Harkness, Browning, & Wagner, 1997). They are one of the world's largest fruit crops with a global production of 68 million tonnes annually of which, 38 million tonnes is processed. Annually around 2.5 million tonnes of grape waste is generated from winery and grape juice industry (Food and Agriculture Organization of the United Nations, 2012; Zhu, Du, Zheng, & Li, 2014; Krishnaswamy, Vali, & Orsat, 2014; Doshi, Adsule, Banerjee, & Oulkar, 2015). Grapes and their seeds contain important vitamins, minerals, lipids, proteins, carbohydrates, and a complex pool of polyphenolic compounds, mainly the catechin monomers, or their dimers, trimers, and oligomers, and are commonly known as proanthocyanidins (Fig. 1) (Monagas, Gomez-Cordoves, Bartolome, Laureano, & Ricardo da Silva, 2003; Weber et al., 2007; Fine, 2000; Jara-Palacios et al., 2014; Teixeira, Baenas, Dominguez-Perles, et al., 2014; Dang, Zhanga, & Xiua, 2014). GSE, an industrial derivative from whole grape seeds is being used as a nutraceutical agent that is commonly consumed as a health/dietary supplement (Kaur, Agarwal, & Agarwal, 2009; Zhu, Du, & Li, 2015). Interest in GSE emerged in the late 20th century with explorations into the "French Paradox". The paradox has expounded how despite having a diet high in saturated fats, the French rate of mortality from heart disease is relatively low (St. Leger, Cochrane, & Moore, 1979). It has recently been reported that GSE has shown wide spectrum of pharmacological and biochemical actions such as its anti-diabetic (Pinent et al., 2004; Lavelli, Sri Harsha, & Fiori, 2015), anti-bacterial (Mayer et al., 2008; Molva & Baysal, 2015), anti-cancer (Kaur, Singh, Gu, Agarwal, & Agarwal, 2006; Zhu, Du, et al., 2015) chemoprotective properties against reactive oxygen species (Nandakumar, Singh, & Katiyar, 2008) and oxidative stress (Gupta & Prakash, 2015; Zhu, Du, et al., 2015) as well as being anti-inflammatory (Terra et al., 2009; Zhu

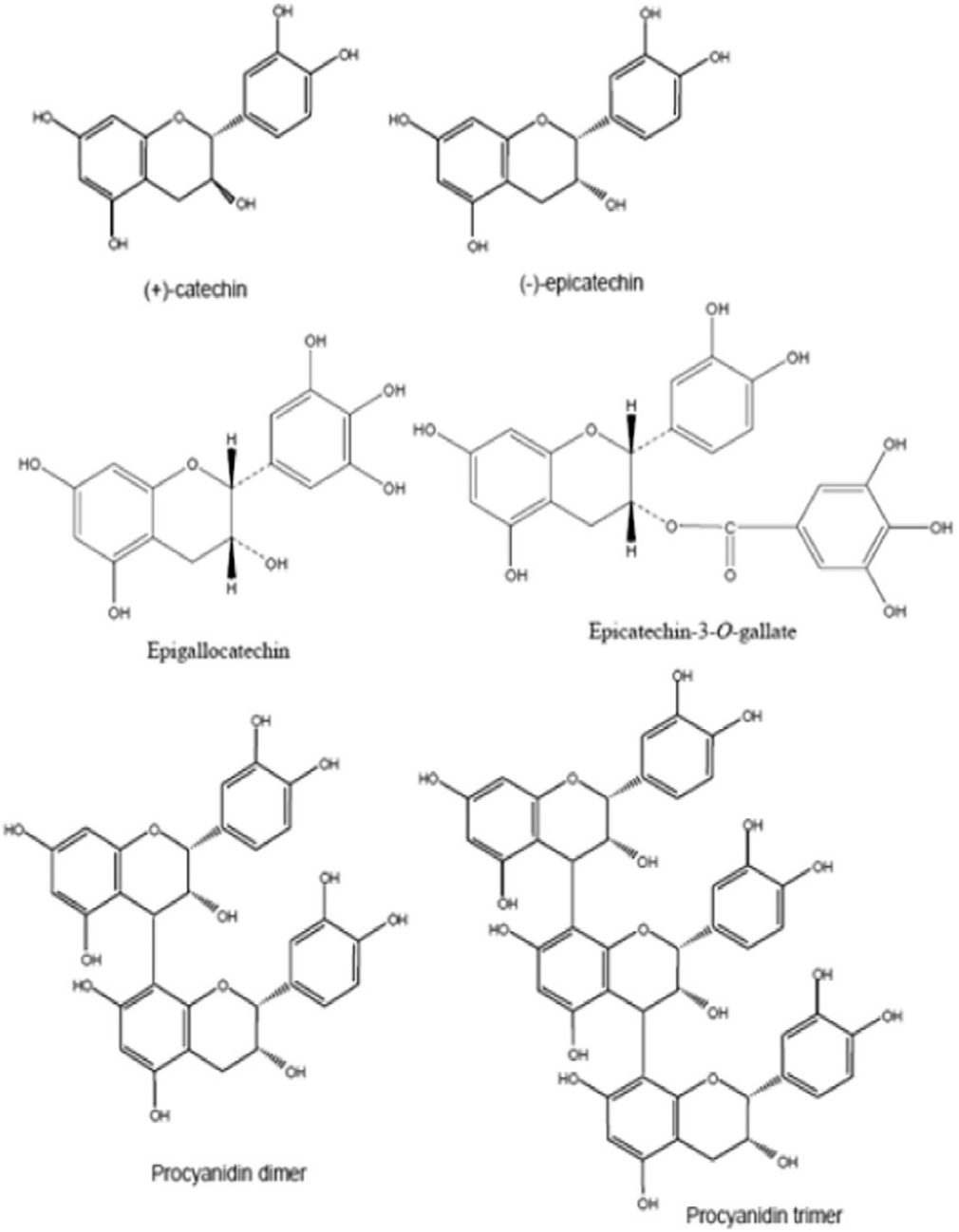

Fig. 1. Chemical structures of phenolic compounds present in grape seeds.

et al., 2015a; Zhu et al., 2015b). In addition, GSE has been found to reduce plasma cholesterol in rabbits, and that may attenuate the risk of atherosclerosis and coronary heart disease (Yamakoshi, Kataoka, Koga, & Ariga, 1999).

In this review, we have focused our discussion on recent furtherance in GSE regarding their health beneficial activities. Commercial preparations of grape seed polyphenols (GSPS) are marketed in the United States as GSE and are sold as an over-the-counter product in the form of capsules or tablets (100–500 mg), with 95% standardized proanthocyanidins as a dietary supplement because of its health benefits, particularly the strong antioxidant activity (Kar, Laight, Rooprai, Shaw, & Cummings, 2009; Escribano-Bailon, Gutierrez-Fernandez, Rivas-Gonzalo, & Santos-Buelga, 1992). The antioxidant capacity of this extract has been shown to be greater than known antioxidants such as vitamin C and E (Bagchi et al., 1997).

2. Chemistry of grape seeds

Grape seed is a complex matrix that consists of 40% fiber, 16% oil, 11% protein and 7% complex phenols and tannins besides sugars, mineral salts etc. (Murga, Ruiz, Beltran, & Cabezas, 2000). Grape seeds also contain flavonoids (4–5%), including kaempferol-3-O-glucosides, quercetin-3-Oglucosides, quercetin and myricetin (Nassiri-Asl & Hosseinzadeh, 2009). The slimy film, that surrounds the seeds are rich in polyphenolic compounds such as monomeric, dimeric, trimeric and tetrameric procyanidins and proanthocyanidins, which are otherwise known as tannins (Silva, Rigaud, Cheynier, & Chemina, 1991; Zhang et al., 2015). These proanthocyanidins are the oligomer and polymer of flavan-3-ol with an average degree of polymerization (DP) ranging from 2 to >15 and an average molecular mass ranging from 578 to >5000 Da (Percival, 2009). The proanthocyanidin monomers found in GSEs are the catechins and epicatechins. Besides the monomers (+)-catechin, (−)-epicatechin and their esters with gallic acid, 14 dimeric, 11 trimeric and one tetrameric procyanidins have been identified in grape seeds (Nassiri-Asl & Hosseinzadeh, 2009; Bautista-Ortin, Busse-Valverde, Lopez-Roca, Gil-Munoz, & Gomez-Plaza, 2014). The proanthocyanidin content in grape seeds is highly dependent on their varieties and extraction procedures. In general, the galloylated procyanidins are present in considerably lower concentrations than the nongalloylated ones (Fuleki & Ricardo-da-Silva, 1997), and higher molecular weight polymers constitute the majority of proanthocyanidins in grape seeds (Prieur, Rigaud, Cheynier, & Moutounet, 1994). Table 1.1 lists most of the polyphenols and phenolic acids found in grape seeds. The distribution of phenolic compounds in the GSE is shown in Table 1.2.

3. Extraction of GSPs

The efficient methods for extracting phenolics especially proanthocyanidins from grape seeds have been of great interest to many investigators, due to the special health promoting and disease preventing effects of polyphenols (Shi, Yu, Pohorly, & Kakuda, 2003). Without proper knowledge of extraction techniques, one cannot

Table 1.1
List of principal classes of polyphenols and phenolic acids found in grape seeds (Santos-Buelga, Francia-Aricha, & Escribano-Bailon, 1995; Dinicola et al., 2014; Teixeira et al., 2014).

Compounds found in grape seeds							
Polyphenols						Hydroxybenzoic and phenolic aids	Still benes
Flavan-3-ols	Flavones	Flavanones	Flavonds	Isoflavones	Anthocyamins		
	Luteolin	Naringenin	Quercetin	Genistein	Delphinidin	Ellagic acid	Reveratrol
(+)-Catechin	Diosmetin	Hesperidin	Myricetin	Daidzein	Pelargonidin	Gallic acid	Trans-reveratrol
(−)-Epicatechin	Chrysin	Eriodictyol	Rutin		Cyanidin	Vanillic acid	
(−)-3-Epicatechinn-3- O -gallate	Wogonin		Kaemoferol		Petunidin	Caffeic acid	
Epicatechin-(4–8)epicatechin (BI)	Apigenin				Malvidin	Coumaric acid	
Catechin-(4–8)-catechin-(4–8)-catechin (C2)						Ferulic acid	
						Gentistic acid	
Epicatechin-(4–8)-epicatechin-(B2)							
Epicatechin-3 - O - gallate-(4–8)-catechin-(B1-3- O -gallate)							
Catechin-(4–6)-catechin(B 6)							
Epjcatechin-(4–8)-epicatechin-(4–8)-epicatechin-3-O-gallate							
Catechin-(4–8)-epicatechin-3-O-gallate(B4- 3′-O-gallate)							
Catechin-(4–8)-catechin-(4–8)-epicatechin							
Epicatechin-(4–8)epicatechin-(4–8)-catechin							
Catechin-(48)-catechin (B 3)							
Catechin-(4–8)-epicatechin (B 4)							
Epicatechin-(4–6)-epicatechin-(4–8)-epicatechin							
Epicatechin-(4–8)-epicatechin-3-O-gallate(4–8)-epicatechin-3-O-gallate							
Epicatechin-3-O-glallate-(4–6)-catechin-(B7-3-O-gallate)							
Epicatechin-(4–8)-epicatechin-3-O-gallate-(4–8)catechin							
Epicatechin-3-O-gallate-(4–8)-epicatechin-(B2-3-O-gallate)							
Epicatechin-(4–6)-epicatechin-(4–6)-catechin							
Epicatechin-(4–8)-epicatechin-(4–8)-epicatechin (C1)							
Epicatechin-3-O-gallate-(4–8)-epicatechin-3-O-galla(B2 -3.3′ -O-digallate)							
Epicatechin-(4–8)-epicatechin-(4–6)-catechin							
Epicatechin-(4–6)-epicatechin (Dimer B5)							
Epicatechin-(4–8)-epicatechin-(4–8)-epicatechin-(4–8)-epicatechin							
Epicatechin-(4–6)-catechin-(B7)							

Table 1.2
Phenolic content in GSE (Robinson et al., 2012).

Total phenol content (gallic acid equivalents, g/100 g)	93.9 ± 0.9
Epicatechin gallate terminal units (%)	0%
Epicatechin gallate extension units (%)	5.7 ± 0.6
Monomers (%)[a]	9.1 ± 1.2
Oligomers (%)[a]	68.7 ± 1.2
Polymers (%)[a]	22.3 ± 0.6
Catechin and epicatechin by weight (%)	9.9 ± 0.6

[a] Determined by reverse-phase HPLC using peak area.

characterize these compounds properly. Due to their variations in size and structure, most current separation methods are designed to not only extract one particular type of proanthocyanidin, but also extract as many different types as the methods can. Traditionally, organic solvents such as methanol, acetone, acetonitrile, ethyl acetate, and others are used to extract polyphenols from grape seeds (Pekic, Kovac, Alonso, & Revilla, 1998; Kallithrake, Garcia-Viguera, Bridle, & Bakker, 1995; Bonilla, Mayen, Merida, & Medina, 1999; Dang et al., 2014). Some early studies showed ethanol (Thorngate & Singleton, 1994) and boiling water (Adams, 1972; Moore, Francis, & Jason, 1982) to be effective for polyphenol extraction from food material. Because polyphenols are polar compounds, the extraction should be completed by water. A US patent (Nasis-Moragher, Svanoe, & Seroy, 1999) described the process of using hot water at high temperature and high pressure to extract procyanidins from grape seeds. However, water alone also dissolves undesired protein and polysaccharide, particularly under high pressure and temperature. Moreover, these techniques require long extraction periods and result in low yields of extract (Duba, Casazza, Mohamed, Perego, & Fiori, 2015). Safety concerns associated with the use of organic solvents for industrial extractions include solvent residues in the product, exposure to workers, disposal of waste solvents, and pollution of the environment.

Nawaz, Shi, Mittal, and Kakuda (2006) described a solvent extraction method using 50% ethanol and 50% water as solvent to extract polyphenols from grape seeds. Ultrafiltration membrane system with average pore size of 0.22 μm was utilized to concentrate extracts. Since the process does not involve any toxic solvent, this approach can be adopted for the use in the food and nutritional supplement industries. Countercurrent chromatography and solid phase extraction can also be used with abovementioned solvents to improve yield and efficiency. All liquid/liquid extraction techniques can be further assisted with microwave, ultrasound, and thermo-mechanical (including extrusion) and enzymatic pretreatment of grape seeds prior to extraction (Li, Wang, Zheng, Fan, & Xie, 2010; Zhang, Sun, Wang, & Tan, 2007; Garcia-Marino, Rivas-Gonzalo, Ibanez, & Garcia-Moreno, 2006; Li, Zheng, Wang, Yang, 2010). Recently, subcritical water (SW) extraction, also referred as pressurized or low polarity water extraction, has been an alternative technique for the extraction of both polar and non-polar compounds from grade seeds (Duba et al., 2015). Furthermore, monomer (+)-catechin; (−)-epicatechin and (−)-epicatechin-3-o-gallate, three oligomeric procyanidin fractions (OPCs) and one polymeric procyanidin fractions (PPCs) have been successfully extracted from GSEs using semi-preparative TBE-300B high-speed counter-current technique (Shuting, Yan, & Baoshan, 2014).

4. Bioavilability of GSPs

Oral absorption and bioavailability of PAs has been extensively studied in both animals and humans in terms of uptake and distribution. The absorption, metabolism and degradation of PAs contribute to PA bioavailability (D'Archivio et al., 2007). The bioavailability of these compounds is generally governed by their chemical structures and DP (Choy & Waterhouse, 2014). Upon ingestion of PAs, these compounds react with proline-rich proteins in the mouth and the oral mucosa to elicit astringent responses (Payne, Bowyer, Herderich, & Bastian, 2009) leaving the remainder to reach the intestinal barrier. Certain forms of PAs (DP < 3) will cross the intestinal barrier and be transported to the liver via the vasculature, where they may reach all tissues within hours following ingestion as reported in radio-labeled experiments with live rats (Gonthier, Donovan, Texier, Felgines, et al., 2003). It has been shown that low molecular weight forms, especially monomeric flavan-3-ols and dimers, are absorbed in the small intestine and metabolized by the phase-II enzymes, whereas the polymeric forms are metabolized by the colonic microbiota (Aura, 2008; Monagas et al., 2010). In addition, after digestion, the metabolized compounds can lose their original properties or even acquire new activities (Margalef et al., 2014). It has also been demonstrated that 2 h after an acute PA administration, the main compounds that reach the systemic circulation and tissues are phase-II metabolites (Serra et al., 2010, 2013). One study using an oral GSE containing 20% and 30% dimers and trimers noted that the circulating levels of dimers and trimers after ingestion equated 0.35% and 0.01%; respectively (Serra et al., 2010). This was due to excessive breakdown of dimers and trimers into monomeric catechins, which elevated from 36% of the GSE by weight to comprise 99% of circulating flavanols (Serra et al., 2010). Deprez, Mila, Huneau, Tome, and Scalbert (2001) tested the absorption of radio-labeled PAs by Caco-2 human colonic cell lines grown on filter membranes. These investigators reported that lower molecular weight PA oligomers (average degree of polymerization = 3) could be absorbed intact across a model of the gastrointestinal tract, while the absorption of larger oligomers (average degree of polymerization = 7) was 10 times lower, suggesting that polymers are not likely to be readily absorbed in vivo. Donovan et al. (2002) fed rats GSE, (+)-catechin and procyanidin B3 meals. While conjugated metabolites of (+)-catechin were detected in plasma and urine after both the (+)-catechin and GSE meals, there was no evidence for absorption of the procyanidins.

5. Biological activities of grape seeds

5.1. Antioxidative activities

Oxidative stress is a hallmark of various health problems. The antioxidant activities of grape seeds have been extensively studied since the finding of the French Paradox (Jeong & Kong, 2004). GSEs have gained significant interest based on the promising results of their antioxidant activities that protect the body from premature aging, disease and decay. The various antioxidants reported in GSEs are polyphenolics. The pharmacological and nutraceutical benefits derived from these GSPs are because of their free radical scavenging capacity. Studies have shown that GSPs attenuated the risk of cancer and heart disease by inhibiting the oxidation of low density lipoprotein (LDL) (Shi et al., 2003). In recent years, the antioxidant potential and health benefits of GSE has lead to its use as a nutritional supplement and food additive and the same has been demonstrated by various in vitro and in vivo studies (Gonzalez-Paramas, Esteban-Ruano, Santos-Buelga, Pascual-Teresa, & Rivas-Gonzalo, 2004; Nakamura, Tsuji, & Tonogai, 2003). Flavonoids reported in GSE have been found to be chain-breaking inhibitors of the peroxidation process which result in chelation of metal ions and scavenging of free radicals (Morel et al., 1993; Tebib, Rouanet, & Besancon, 1997; Yilmaz & Toledo, 2004). Grape seeds have been found to neutralize free radicals which are believed to contribute in the development of a number of health related problems like cancer and heart diseases, and reduce or even help in preventing the damage caused by these radicals because of their potent antioxidant properties (Sovak, 2001). Grape seed proanthocyanidin extract (GSPE) provided significant protection than vitamin C, E and β-carotene against free radicals and free radical-induced lipid peroxidation and DNA damage in liver and brain tissues using similar doses (Bagchi et al., 1997). Similarly, a clinical report has shown that antioxidant potential of proanthocyanidins from grape seeds are 20 times greater than vitamin C and 50 times greater than vitamin E (Uchida, 1980). In vitro studies have demonstrated that monomeric and polymeric grape seed proanthocyanidins (GSP) exhibit potent scavenger capacity for

superoxide and hydroxyl radicals (Ricardo da Silva, Darmon, Fernandez, & Mitjavila, 1991) and can greatly inhibit the oxidation of LDL and lipid-containing membranes induced by metal ions or radical generators (Mazur, Bayle, Lab, Rock, & Raysiguier, 1999; Teissedre, Frankel, Waterhouse, Peleg, & German, 1996). An in vivo study done by Yamakoshi et al. (1999) on GSPE (270 mg/kg/day) has also shown to prevent oxidation of LDL and, therefore, attenuated the development of aortic atherosclerosis in cholesterol fed rabbits after an 8 week feeding trial. Morin, Narbonne, Ribera, Badouard, and Ravanat (2008) observed that GSPE has protective activity against oxidative DNA damage in leukocytes of healthy rat subjects.

GSE administered to female mice exhibits higher protective ability than vitamin E, C, vitamin E plus C, and β-carotene against 12-*O*-tetradecanoylphorbol-13-acetate-induced lipid peroxidation and DNA fragmentation in the hepatic and brain tissues, as well as peritoneal macrophage activation (Bagchi et al., 1998). In vivo studies using GSE at a dose of 600 mg/day for 4 weeks significantly improved glutathione (GSH)/oxidized glutathione and total antioxidant status, and reduced oxidative stress in a double-blinded randomized crossover human trial (Kar et al., 2009). In high-cholesterol human subjects GSE supplementation (2 × 300 mg/day) was found to ameliorate plasma antioxidant capacity and lipid profile in hypercholesterolemic subjects (Vinson, Proch, & Bose, 2001). Moreover, several studies have observed the antioxidative capacity of GSE by β-carotene linoleate model system and linoleic acid peroxidation method (Jayaprakasha, Singh, & Sakariah, 2001) as well as by phosphomolybdenum complex and diphenyl picryl hydrazyl methods (Jayaprakasha, Selvi, & Sakariah, 2003). In vitro study done by Furiga, Lonvaud-Funel, and Badet (2009)compared GSE with ascorbic acid and chlorhexidine for their capacity to quench chemically generated 2,2-azino-bis 3-ethyl benz thiazoline-6-sulfonic acid cation radicals. Under the experimental conditions, GSE presented a high Trolox equivalent antioxidant capacity value, proving its capacity to scavenge the 2,2-azino-bis 3-ethyl benz thiazoline-6-sulfonic acid cation radicals. Furthermore, GSPE at a dose of 100 mg/ml provides greater protection against smokeless tobacco-induced oxidative stress and apoptotic cell death in a primary culture of human oral keratinocyte cells as compared to vitamins E and C combination (Bagchi, Bagchi et al., 1999). An in vitro study has showed that addition of 2 mg/l GSP to a system containing polyunsaturated fatty acids and mice liver or brain microsomes reduces the oxidation of arachidonic and docosahexaenoic acids significantly upon oxidation induced with UV-C irradiation (Bouhamidi, Prevost, & Nouvelot, 1998).

By using Langendorff isolated rat heart preparation, GSPE has been found to provide cardioprotective properties by acting as in vivo antioxidant and by virtue of its ability to scavenge both the hydroxyl and peroxyl free radicals formed during ischemic reperfusion (Sato, Maulik, Ray, Bagchi, & Das, 1999). It is also observed that GSE prevents cardiac cells from cell death via activation of endogeneous antioxidant enzymes (Du, Guo, & Lou, 2007). In a recent study, it has been demonstrated that administration of GSPE at a dose of 100 mg/kg/day for 3 weeks reduces the production of the reactive oxygen species (ROS) and inhibits the expression of proapoptotic transcription factor JNK-1 and gene c-Jun., thereby producing cardioprotective effect in rats with ischemic/reperfused hearts (Sato et al., 2001). Procyanidins from grape seeds have also been found to increase the total antioxidant plasma capacity and the ascorbic acid plasma level in in vitro models (Maffei Facino et al., 1998). In addition, Ricardo da Silva et al. (1991)found that monomeric and polymeric GSP have superoxide and hydroxyl radicals scavenging activity. Comparing the superoxide and hydroxyl radical scavenging abilities of these compounds with trolox the study concludes that epicatechin, epicatechin polymers and the B procyanidins are more potent antioxidants than catechin and catechin polymers. More interestingly, in vivo antioxidant activity in rats fed with a diet high in cholesterol and deficient in vitamin E but supplemented with grape seed tannins was also assessed. The polymeric grape seed tannins in the diet have been found to increase the levels of enzymatic antioxidants such as catalase, glutathione peroxidase and superoxide dismutase in different tissues. In addition, the polymeric grape seed tannin supplementation effectively restored the total glutathione level in blood and reduced lipid peroxidation in plasma and heart, liver and intestinal mucosa tissues as effectively as vitamin E (Tebib et al., 1997). In yet another study conducted by Enginar, Cemek, Karaca, and Unak (2007) the antioxidant effect of GSE supplementation given to animals has been reported to enhance the levels of GSH, retinol, β-carotene and ceruloplasmin concentration, and decrease the level of malondialdehyde (MDA) and nitrite concentration in blood samples of rats exposed to X-radiation. However, this antioxidant effect of GSE has been more than vitamin E, administered before whole-body irradiation in rats.

El-Ashmawy, Saleh, and Salama (2007) reported that co-administration of GSE along with Marjoram volatile oil attenuated the oxidative damages and resulted in minimizing the hazardous effects of ethanol induced toxicity on male fertility, liver, and brain tissues of rats. Similarly, the oxidative DNA damage in human cultured cells and isolated DNA is prevented by GSPE by decreasing the formation of 8-oxodG (Sakano et al., 2005). Ahn et al. (2002a)and Ahn et al. (2002b) observed that GSE feeding increased superoxide dismutase and catalase activities in liver tissue at 0.5 mg/ml concentration and exhibited radical scavenging activity of 94.87% when evaluated by Chemiluminescence assay. Furthermore, it is also reported that GSEs have been associated with other antioxidants to accelerate the prevention of oxidation reactions (Lu & Foo, 1999). The antioxidant efficacy of GSE in various meat products has also been evaluated for their use as food lipid antioxidants. During the experimental studies, it has been observed that GSE acts by means of inhibiting the lipid oxidation and rancidity in meat products such as cooked beef (Ahn et al., 2002b). In addition, GSE supplementations (10 g and 20 g GSE/kg of turkey patties) have been found to down-regulate 10 times the thiobarbituric acid reactive substances as compared to the product without having GSE supplement (Lau & King, 2003). Luther et al. (2007) investigated the inhibitory effect of GSE on lipid peroxidation in fish oil. The authors came to the conclusion that lipid peroxidation of total polyunsaturated fatty acids, including 18:3n − 3 (α-linolenic acid), linolenic acid, 20:5n − 3 (eicosapentaenoic acid) and 20:4n − 6 (arachidonic acid) were inhibited when the fish oil contained 16.7 mg of GSE. In addition, GSE significantly reduced 2,2-diphenyl-1-picrylhydrazylradical and oxygen radical absorbance capacity values compared to control. Grape seed polyphenolic extract at a concentration of 2000 ppm in refined poppy seed oil has been tested for its capacity to scavenge hydrogen peroxide and to decrease the rate of peroxide formation, it was observed that grape seed polyphenolic extract was more potent than butylated hydroxytolune and butylated hydroxyanisole (Baydar, Ozkan, & Yasar, 2007).

The consumption of grape-derived dietary flavonoids in the form of grape extracts and grape seed powders has been shown to effectively suppress oxidative stress and prevent oxidative damage in vivo (Georgiev, Ananga, & Tsolova, 2014). Grape seed tannins presented a higher antioxidant activity than the apple ones (G15ox > A6 > A15ox) measured by the CAT test (Figueroa-Espinoza, Zafimahova, Alvarado, Dubreucq, & Poncet-Legrand, 2015). The antioxidant effect of GSE against Ehrlich solid tumor (Est)-induced oxidative stress, hepatic dysfunction and pathological changes in the liver of albino mice has been investigated. GSE supplementation resulted in the amelioration of liver function enzymes, reduced MDA level, augmented antioxidant parameters, normalized liver protein and DNA contents and improved the pathologically examined hepatic lesions (Ali, Badr El-Din, & Abou-El-magd, 2015).

5.2. Antidiabetic activity

It is worth nothing that there are 382 million of people worldwide suffering from diabetes, but this figure could rise to 592 million by the year 2035 (International Diabetes Federation, 2013). Hence, there is a need to develop new dietary strategies that efficiently target prevention of this chronic disease especially becoming common among aging

populations (Lavelli et al., 2015). GSE and its bioactive constituents exhibit favorable anti-diabetic activities by stimulating glucose uptake in insulin-sensitive cell lines, decreasing hyperglycemia in streptozotocin (STZ)-induced diabetic rats (Pinent et al., 2004) and inhibiting intestinal pancreatic α-amylase, α-glucosidase activities and the process of glycation which results in delayed carbohydrate digestion of absorbable monosaccharide (Adisakwattana et al., 2010). Rats fed with a high-fructose diet supplemented with 1% GSE significantly reduce plasma glucose and insulin concentrations, indicating that GSE improves glucose tolerance and prevents insulin resistance in high-fructose induced diabetic rats (Suwannaphet, Meeprom, Yibchok-Anun, & Adisakwattana, 2010). Another study has shown that diabetic rats supplemented with GSE at a dose of 100 mg/kg/day increases the paraoxonase activities in control and diabetic rats, but this effect has been found to be more in the diabetic population, who are more prone to atherosclerotic events compared to the healthy population (Kiyıcı, Okudan, Gokbel, & Belviranli, 2010). A recent double blind randomized trial has demonstrated that daily dietary supplementation with 600 mg GSE for 4 weeks significantly improves markers of glycemia in obese Type 2 diabetic subjects (Kar et al., 2009). It has also been demonstrated that HF-fed rats supplemented with GSE substantially attenuated insulin resistance, hyperinsulinaemia and hypertriacylglycerolaemia in rats by improving the expression of insulin signaling pathway-related proteins, including Akt and glucose transporter-4. In addition, it also increased the mRNA expression of adiponectin, AdipoR1 and AMPK-a (Meeprom, Sompong, Suwannaphet, Yibchokanun, & Adisakwattana, 2011).

In vitro study has found that GSPE treatment to insulin-resistant 3T3-L1 adipocytes produces higher stimulating capacity for glucose uptake compared to insulin (Montagut et al., 2010). In one of the studies, it has been clearly demonstrated that oligomers of GSPE activate the insulin receptor thereby stimulating the uptake of glucose related to phosphorylation of Akt, p44/42 and p38 mitogen-activated protein kinases (MAPKs), their activation however differs from insulin activation and results in differences in downstream signaling (Montagut et al., 2010). El-Alfy, Ahmed, and Fatani (2005) demonstrated that oral administration of 50 to 100 mg/kg body weight of GSP to alloxan-induced diabetic rats for up to 72 h has been found to ameliorate the damage to pancreatic tissue by alleviation of oxidative stress. Exercise training (ET) combined with GSE administration has been reported to restore endothelial dependent and independent dysfunction of coronary vascular bed of STZ-induced diabetic rats more significantly than exercise training or GSE alone (Badavi, Abedi, Sarkaki, & Dianat, 2013). Furthermore, GSE treatment attenuates the radiation-induced oxidative damage in pancrea tissues substantiated by a significant amelioration in radiation-induced hyperglycemia and hyperinsulinemia (Saada, Said, Meky, El, & Azime, 2009). GSPE has been found to contribute in the prevention and treatment of diabetic vascular complications by means of reducing the advanced glycation end products (AGE)-induced receptor for AGE expression through upregulating peroxisome proliferators-activated receptor γ expression in human endothelial cells of patients with diabetes (Ma et al., 2007). Most recently, Ding et al. observed that GSPEs ameliorated oxidative stress and endoplasmic reticulum stress to protect skeletal muscle from cell death in a low-dose STZ- and HF-induced diabetic model (Ding, Dai, et al., 2013). Another study of similar nature has shown that GSP treatment partially reverses the pancreatic β-cell dysfunction and the abnormal oral glucose tolerance in low-dose STZ- and a high-carbohydrate/HF-induced diabetic rats. Moreover, GSP treatment increases normal insulin content by means of alleviating endoplasmic reticulum stress through inhibition of some endoplasmic reticulum stress markers in diabetic pancreas (Ding, Zhang, et al., 2013). In addition, findings from a study has reported that GSPE administration significantly decreases lipid peroxidation, increases renal antioxidant enzyme activity and reduces kidney weight thereby ameliorating diabetic nephropathy in rats without having any effect on body weight (Mansouri, Panahi, Ghaffari, & Ghorbani, 2011).

Pinent et al. have discovered that GSPE is having similar anabolic properties to insulin but at the same instance is less efficient at activating glycogen metabolism. Moreover, the differences between the effects of GSPE and insulin has indicated that GSPE uses a mechanism complementary to that of insulin to induce its insulinomimetic properties in 3T3-L1 adipocytes (Pinent, Bladea, Salvadoa, Arola, & Ardeavol, 2005). In an animal model, Decorde et al. (2009) reported that supplementation of grape seed polyphenolic extract for 12 weeks resulted in reducing obesity development, lowering insulin resistance, preventing hyperglycemia and hyperinsulinemia, and improving adiponectin secretion in hamsters fed a HF diet. It is also reported that GSP provide protective activity against high glucose-induced cytotoxicity (Fujii, Yokozawa, Kim, Tohda, & Gen-ichiro, 2006). Administration of GSPE at a dose of 250 mg/kg/day for 24 weeks has been found to upregulate the expression of catalase and apolipoprotein A-I in diabetic rats which resulted in reducing the aortic lesions in diabetes mellitus (Li et al., 2009a; Li et al., 2009b). Similarly, GSPE has also been reported to be effective in treating diabetic nephropathy, a major cause of morbidity and mortality in diabetic patients. Out of the twenty-five proteins found in the kidneys of untreated diabetic rats only nine proteins which are involved in oxidative stress, glycosylation damage, and amino acid metabolism were found to be back-regulated to normal levels after GSPE therapy (Li et al., 2008a; Li et al., 2008b). Another study done on animal model by Zhou et al. (2005) have observed that GSPE administration downregulated the formation of AGEs in STZ-induced diabetic rats which in turn are responsible for myocardial damage; while as fibrosis and inflammation are associated with accelerated diabetic cardiomyopathy (Goldin, Beckman, Schmidt, & Creager, 2006; Zhang et al., 2006a; Zhang et al., 2006b). Similar results have been reported by Li et al. (2009a) and Li et al. (2009b) in STZ-induced diabetic rats, GSE administered at a dose of 500 mg/kg/day for a period of 24 weeks has been able to reduce the levels of AGEs and the receptor for AGEs, a mechanism also observed in cardiac (Cheng et al., 2007) and neural tissue (Lu et al., 2010). Furthermore, it has been proven that GSPE downregulates the expression of vascular cell adhesion molecule-1 surface proteins and mRNA induced by AGEs in endothelial cells through its potent antioxidant activity thereby interfering with chronic diabetic complications (Zhang, Gao, et al., 2006).

In a recent study, oral administration of GSE (100 mg kg^{-1} day^{-1}) for 6 weeks exerted a protective effect on oxidative stress and antioxidant status in STZ-induced diabetic rats (Belviranli, Gökbel, Okudan, & Büyükbaş, 2015). It has been observed that GSE decreases oxidative stress and neuronal apoptosis occurring in the hippocampus of streptozotocin-induced diabetic rats. In addition to this it also demonstrated that while TNF-α and NF-κB expression was increasing in the hippocampus of diabetic animals, GSE treatment decreased the expression of both TNF-α and NF-κB (Yonguc et al., 2015). Recently a study done by Chen et al. (2015) showed that GSPE could ameliorate diabetic bladder dysfunction and decrease the apoptosis of the bladder in diabetic rats, a finding that may be associated with its antioxidant activity and ability to activate the Nrf2 defense pathway (Chen et al., 2015).

5.3. Anticancer activity

The World Cancer Research Fund has estimated that up to one-third of the cancers that occur in developed countries like the United States are related to overweight or obesity, physical inactivity, and/or poor nutrition. Fruits and vegetables have been hypothesized to be major dietary contributors to cancer prevention (Olaku, Ojukwu, Zia, & White, 2015). GSEs have received much attention for their potential anticancer properties. It is reported that topical treatment of GSE containing mainly oligomeric and polymeric proanthocyanidins has been found to inhibit 12-*O*-tetradecanoylphorbol-13-acetate-induced tumor promotion in 7,12-dimethylbenz[a]anthracene initiated CD-1 mice skin (Bomser, Singletary, Wallig, & Smith, 1999). As high levels of ornithine decarboxylase are associated with increased risk for cancer, polyphenolic

fraction isolated from grape seeds is also shown to inhibit epidermal ornithine decarboxylase activity in mice when used before 12-*O*-tetradecanoylphorbol-13-acetate (Bomser, Singletary, & Meline, 2000). In vivo animal experiments have shown that dietary feeding of GSP to SKH-1 hairless mice has significantly resulted in inhibition of UV radiation–induced skin carcinogenesis in terms of tumor incidence, tumor multiplicity, and tumor volume (Mittal, Elmets, & Katiyar, 2003). Results have shown that topical application of GSP at doses of 0.1 and 1.5 mg/mouse/application to the dorsal initiated SENCAR mouse skin results in anticarcinogenic effects in 7,12-dimethylbenz[a]anthracene initiated and 12-*O*-tetradecanoylphorbol-13-acetate promoted skin tumorigenesis possibly due to increase in their antioxidant activity, as evidenced by a significant reduction in tumor occurrence and tumor volume (Zhao, Wang, Chen, & Agarwal, 1999). It has also been researched that oral feeding of 1% (*w*/*w*) GSE in the diet to MIN mice inhibits adenomatous polyposis coli mutation-associated intestinal adenoma formation (Arii et al., 1998). Another study has reported that GSE has a possible role in antiproliferation and apoptosis of human prostate carcinoma (PCA) as it inhibits mitogenic signaling in PCA DU145 cells and activates JNK, a protein kinase associated with the apoptosis response (Tyagi, Agarwal, & Agarwal, 2003). Recent in vitro cell culture studies have shown that GSPE exhibits anti-cancer efficacy against several malignancies of different anatomical origin including human breast carcinoma MCF-7, human lung cancer A-427, and human gastric adenocarcinoma CRL-1739 cells in a concentration- and dose-dependent manner while enhancing the growth and viability of normal cells (Ye, Krohn, Liu, et al., 1999). Moreover, with regard to the in vivo efficacy of GSE against PCA, it has been reported that oral feeding of GSE inhibits prostate tumor growth and angiogenesis (Singh, Tyagi, Dhanalakshmi, Agarwal, & Agarwal, 2004). Park et al. (2011) have demonstrated the protective effect of GSE against PCA by means of regulating the androgen receptor-mediated transcription in LNCaP cells through significant anti-histone acetyltransferase activity. More recently, the oral feeding of GSE (200 mg/kg body weight) to transgenic adenocarcinoma of the mouse prostate model resulted in decreased incidence of prostatic adenocarcinoma and decreased tumor growth and progression in mice (Raina, Singh, Agarwal, & Agarwal, 2007). Another study done by Veluri et al. (2006) have identified gallic acid as a major constituent in GSE at inducing growth inhibition and apoptotic death of human PCA DU145 cells in culture. Furthermore, proanthocyanidin-rich GSE targeted the transcription factor, nuclear factor kappa B (NF-κB) by inhibiting its DNA-binding capacity and downregulate urokinase plasminogen activator in highly metastatic androgen-independent PC3 PCA cells and, therefore, inhibits cell invasion (Uchino, Madhyastha, Madhyastha, et al., 2010).

In regards to anti-cancer effects in the colon, GSE was able to induce growth inhibition and apoptosis in both Caco2 as well as in HCT-8 colon cancer cells. These inhibitive effects were significantly greater than those recorded with isolated epigallocatechin, GSP and the association of both, suggesting a potential additive or synergistic effect among the grape seed components (Dinicola et al., 2012). GSE (25–100 μg/ml) has also been found to induce cell apoptosis through the induction of early apoptosis-inducing factor and enhancement of caspase-dependent apoptosis in human colon cancer cell line Caco-2 (Dinicola et al., 2010). Similarly, Radhakrishnan, Reddivari, Sclafani, Das, and Vanamala (2011) found that GSE in combination with resveratrol, a prominent grape skin component, augment apoptotic effect of GSE in colon cancer cells, which is strongly correlated with p53 levels and Bax:Bcl-2 ratio. Moreover, in a rat dual-organ tumor model, GSP inhibited azoxymethane induced aberrant crypt foci formation (a precursor lesion for colon cancer) and ornithine decarboxylase activity in the distal third of the colon (Singletary & Meline, 2001). Kaur et al. (2006) have evaluated the chemopreventive effect of GSE containing 89% *w*/*w* procyanidins at 25 to 50 μg/ml against colorectal cancer cell lines, HT29 and LoVo cells. During the investigation it has been found that GSE halts the growth of these cancer cells and, more importantly inhibits the growth of HT-29 cells in culture as well as when grown as tumor xenografts in athymic nude mice. Studies investigating the effect of grape seeds on breast cancer also show promising results. Dietary supplementation of GSPE with a dry diet has been reported to inhibit the development of carcinogen induced breast cancer in Sprague–Dawley rats, but no effect has been observed when GSPE was administered with sesame oil, suggesting that whether or not a compound is chemopreventive may depend upon the diet in which the agent is administered (Kim et al., 2004). In addition, GSE oligomers causes irreversible growth inhibition of breast carcinoma MDA-MB468 cells by inhibiting constitutive activation of MAPK/ERK1/2 and MAPK/p38 and causing an induction of CDKI Cip1/p21 and a decrease in CDK4 (Agarwal, Sharma, Zhao, & Agarwal, 2000). GSE (300 mg/kg/day) has also been found to induce accumulation of intracellular ROS in human head and neck squamous cell carcinoma Detroit 562 and FaDu cells, thereby triggering DNA damage and simultaneously causing decrease in DNA repair enzymes. These actions of GSE result in cell cycle arrest in G2/M phase together with cell growth inhibition and apoptotic cell death in human head and neck squamous cell carcinoma cells (Shrotriya, Deep, Gu, et al., 2012).Cheah et al. (2009) have observed recently that rats administered GSE (400 mg/kg) by orogastric gavage reduced intestinal damage in a model of chemotherapy-induced mucositis and afforded protection against 5- Fluorouracil chemotherapy damage in normal intestinal cell lines (IEC-6). GSP has been also reported to inhibit human melanoma A375 and Hs294t cells in a concentration-dependent inhibition of invasion or cell migration of these cells, which resulted in the reduction of prostaglandins E2 synthesis and reversal of epithelial-to-mesenchymal transition (Vaid, Singh, & Katiyar, 2011). Furthermore, Leifert and Abeywardena (2008) have reported that GSE prominently inhibit cell proliferation in cancer cells, and at the same time raised the level of caspases-mediated apoptosis.

In addition, GSE suppresses Akt-related effects on CREB, NFkB, BAD and Bcl-2, thus promoting an overall pro-apoptotic effect on cancer cells (Dinicola, Cucina, Antonacci, & Bizzarri, 2014). Treatment of human pancreatic cancer cells with GSPs has been found to significantly reduced cell viability and induced apoptosis in a dose- and time-dependent manner (Prasad & Katiyar, 2013). GSE efficacy in inhibiting colorectal cancer metastasis to lung in rats further supports its translational potential in controlling colorectal cancer growth, progression and metastasis in patients (Molly, Komal, Rajesh, & Aganwal, 2014). High concentrations (50–400 μg/ml) of GSE has been found to inhibit dose-responsively the proliferation of oral cancer Ca9-22 cells but low concentrations (1–10 μg/ml) of GSE showed a mild effect in a MTS assay. Additionally, high concentrations of GSE dose-responsively induced more γH2AX-based DNA damage than low concentrations. These findings suggest that differential concentrations of GSE may have a differentially antiproliferative function against oral cancer cells (Yen et al., 2015). Moreover, investigating GSE efficacy in inhibiting colo-rectal cancer (CRC) metastasis to lung in an *in situ* pre-clinical animal model supports its translational potential in controlling CRC growth, progression and metastasis in patients (Derrya, Rainaa, Agarwala, & Agarwala, 2014). Selected epidemiological studies have been examined from 2002 to 2009 on the efficacy of total flavonoids, flavan-3-ols, and proanthocyanidins in prostate cancer. Inverse trends were reported with higher total flavonoids and proanthocyanidins with high-grade prostate cancer, but not with advanced prostate cancer (Bagchia, Swaroop, Preussc, & Bagchi, 2014). In a recent study researchers identified procyanidin B2 3,3″-di-O-gallate as the most active constituent of GSE for efficacy against prostate cancer. Both B2 3,3″-di-O-gallate preparations decreased clonogenicity, inhibited cell growth and induced cell cycle arrest and apoptotic death, comparable to each other, in various human prostate cancer cell lines (Zhu, Du, et al., 2015). Moreover, GSE provides a new therapeutic option to decrease the symptoms of intestinal mucositis while concurrently impacting on the viability of colon cancer cells (Cheah, Howarth, & Bastian, 2014).

5.4. Cardiovascular protective action

Oxidative stress, free radicals, smoking and high cholesterol directly leads to the onset of cardiovascular diseases, such as ischemic heart disease, atherosclerosis and cardiomyopathy. Proanthocyanidins from grape seeds are being employed as active ingredients in various medicinal products for the treatment of cardiovascular disorders such as peripheral chronic venous insufficiency, capillary fragility and microangiopathy of the retina (Flamini, 2003). In a study investigating the potential cardioprotective effects of GSEs, it has been reported that proanthocyanidin rich GSEs play a major role in the inhibition of LDL oxidation by acting as antioxidants, and are, therefore, responsible for their cardioprotective activity (Manthey, Buslig, & Baker, 2002). In vitro studies have shown that GSPs are able to slow down LDL-cholesterol oxidation, influence prostaglandin synthesis, stimulate nitric oxide (NO) production and inhibit platelet aggregation (Van de Wiel, 2002). Yu, Wang, Zhao, and Xu (2002) demonstrated that C57BL/6J mice fed with GSE (200 mg/kg, 600 mg/kg) showed a significant increase in serum NO levels, reduction in plasma oxidized LDL and intercellular adhesion molecule-1 levels, and lower thickness of aortic valve than those in model group. These findings indicate that GSE has an inhibitory effect on atherosclerosis. The protective ability of GSPE evaluated against myocardial ischemia–reperfusion injury has been found to reduce the infarct size and improve recovery of post-ischemic contractile functions. It has also been observed that myocardial infarction rate gets decreased in animals fed with GSPE than the control group, and this effect of GSPE has been associated by virtue of its ability to directly scavenge ROS including hydroxyl and peroxyl radicals (Sato et al., 1999). Sato et al. (2001) have demonstrated that rats fed with 50–100 mg/kg/day of GSPE reduced the incidence of cardiomyocyte apoptosis by inhibiting the expression of JNK-1 factor and the c-Jun. gene, which are proapoptotic factors in the ischemic/reperfused myocardium. Another study demonstrated that GSP supplementation in animal models, made the heart less susceptible to the ischemia/reperfusion damage, and significantly raised the vitamin E plasma level and the total antioxidant plasma capacity (Maffei Facino et al., 1996, 1998). Similarly, the cardioprotective activity of GSPE (100 mg/kg) was investigated against myocardial ischemia/reperfusion injury. It has been observed that GSPE improved the recovery of post-ischemic function in isolated rat hearts, due to its ability to reduce free radicals by 75% ± 7% compared to the control (Pataki et al., 2002). Furthermore, administration of GSPE offers an alternative approach to therapeutic cardioprotection against ischemia/reperfusion injury by attenuating ischemia/reperfusion-induced cell death, restoring contractility, enhancing NO production and increasing Akt-endothelial nitric oxide synthase signaling (Shao et al., 2009).

Tebib, Besana, Besanacon, and Rouanet (1994) demonstrated that GSPE fed at 2% of the diet for up to 9 weeks to rats have a pronounced antihypercholesterolemic effect. They attributed this effect of GSPE due to enhanced reverse cholesterol transport, inhibition of intestinal cholesterol absorption, and increase in fecal cholesterol and bile acid excretions which were dependent on the degree of polymerization of procyanidins in GSE. Moreover, it has also been verified that GSEs prominently decreased in vitro cholesterol uptake by up to 66% by inhibition of specific cholesterol uptake/transporters such as the Niemann–Pick C1-like 1 cholesterol transporter, resulting in preventing the development of chronic degenerative diseases like cardiovascular disease (Leifert & Abeywardena, 2008). The GSPE supplementation in a dose of 50 and 100 mg/kg/day orally for 10 weeks to hypercholesterolemic hamsters reduced the atherosclerosis (percentage of aorta covered with foam cells) by 50 and 63%. Moreover, GSPE attenuated plasma total cholesterol and triglyceride (TG) levels, and resulted in a pronounced effect on the oxidative lipid damage as demonstrated by the formation of thiobarbituric acid reactive substances(Vinson, Mandarano, Shuta, Bagchi, & Bagchi, 2002). A recent study has found that 1% GSPE (*w*/*w*) attenuated plasma cholesterol in rabbits fed with a high-cholesterol diet, and this decrease was probably related to the capacity of GSPE to trap ROS in aqueous fluids such as plasma and interstitial fluid of the arterial wall. During this study, it was also found that a diet with 1% (*w*/*w*) of catechin showed very weak anti-atherosclerotic activity in cholesterol-fed rabbits compared with GSPE, suggesting that protective activity of GSEs against atherosclerosis comes mostly from proanthocyanidins (Yamakoshi et al., 1999). Bagchi et al. (2003) reported that GSPE (200 mg/day orally) supplementation given to hypercholesterolemic subjects for 8 weeks significantly improved cardiac function through a wide variety of mechanisms in animal models of cardiac disease including reduced infarct size, reduced ventricular fibrillation and ventricular tachycardia, decreased the amount of ROS, attenuated oxidized LDL and reduced MDA formation in the heart perfusate. Sano, Oda, and Yamashita (2005) assessed the efficacy of GSP on acute coronary thrombotic events. It was found that GSP (20 mg/kg, intravenously or 2 × 200 mg/kg, orally) inhibited the laser-irradiation induced thrombus formation in the carotid artery in mice, confirming its anti-thrombotic effects. GSPE in combination with Doxorubicin has been reported to have a protective effect against Doxorubicin induced toxicity in cardiomyocytes. The cultured primary cardiomyocytes treated with GSPE (50 μg/ml) and Doxorubicin (10 μM) for 24 h, resulted in decreased intracellular ROS production, reduced significant cell death, and increased the redox ratio of reduced GSH/oxidized glutathione (Li et al., 2010a; Li et al., 2010; Li, Zheng. et al., 2010). Moreover, the daily supplementation with 400 mg/day of GSE for 8 weeks exhibited positive results for platelet function in postmenopausal women. It was found that GSE for 8 weeks increased adenosine diphosphate-collagen-stimulated platelet closure time (Shenoy, Keen, Kalgaonkar, & Polagruto, 2007). A randomized controlled study has indicated that blood pressure in persons with metabolic syndrome, when treated with GSE 150 mg/day or 300 mg/day for 4 weeks significantly reduced both systolic and diastolic blood pressures with no prominent changes in serum lipids or blood glucose values as compared with placebo (Sivaprakasapillai, Edirisinghe, Randolph, Steinberg, & Kappagoda, 2009). Research by Peng et al. (2005) indicated that proanthrocyanidins in GSPs reduce salt-sensitive hypertension in young, estrogen-depleted spontaneously hypertensive female rats by about >10 mm Hg. These findings point out the fact that the beneficial effects of GSPs on arterial blood pressure are probably due to their ability to decrease superoxide production by 23% in vivo. A well-characterized GSE, with phenolic compounds forming 93% of its constituents has been reported to elicit maximum endothelium-dependent relaxation (71.9 ± 1.0%) as compared to acetylcholine (64.2 ± 1.5%) in the rabbit aorta. The endothelium dependent relaxation induced by the GSE was found to be mediated by activation of the PI3K/Akt signaling pathway through a redox-sensitive mechanism, resulting in phosphorylation of endothelial nitric oxide synthase (Edirisinghe, Burton-Freeman, & Tissa, 2008). In a fully randomized study on humans, GSE in a dose of 2 g/day favorably alters vascular function, endothelial function, degree of oxidative damage, and significantly improved flow-mediated dilatation in comparison to a control yoghurt (Clifton, 2004).

A recent study has shown that a single combined formulation of GSE and zinc containing multivitamin-mineral nutritional food supplement tablets (Zincovit) can offer a better add-on/substitute drug for statins in hyperlipidemic conditions (Satyam, Bairy, & Pirasanthan, 2014). Another investigation done by Badavi, Abedi, Dianat, and Sarkaki (2015) showed that combination of GSE and exercise training has more substantial improving effects on left ventricular dysfunction in STZ-Induced diabetic rats compared to exercise training or GSE alone (Badavi et al., 2015). Moreover, a study has shown that Chardonnay grape seed flour ameliorate high- fat (HF)-and high cholesterol-induced metabolic disorders (Kim et al., 2015).

5.5. Hepatoprotective activity

The therapeutic efficacy of GSE at a dose of 50 mg/kg/day orally for 28 days has been established against bile duct ligation-induced hepatic

fibrosis in rats. This effect possibly involves the inhibition of neutrophil infiltration and subsequent activation of inflammatory mediators that induce lipid peroxidation, thus restoration of oxidant and antioxidant status in the tissue. Moreover, GSE treatment significantly decreased elevated Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and plasma tumor necrosis factor-alpha levels after the tissue damage (Dulundu et al., 2007). The exposure of rat hepatocytes with defatted milled grape seed extract significantly reduces the oxidant action of adriamycin and preserved the hepatocellular GSH and ATP levels. These results lead to the conclusion that defatted milled grape seed treatment considerably lowered the formation of thiobarbituric acid reactive substances and carbonyl groups in hepatocytes and this effect was most probably due to the phenolic composition of defatted milled grape seed and their antioxidant activity (Valls-Belles, Torres, Beltran, & Martinez-Alvarez, 2006). Ray, Kumar, and Bagchi (1999) reported that GSPE administered at a dose of 100 mg/kg by oral gavage for 7 days to mice attenuated acetaminophen-induced hepatocellular apoptosis and necrosis, and several other acetaminophen-induced toxicity related events at the cellular, subcellular, and molecular levels, when compared to a short-term 3-day exposure with GSPE. Another study done by Joshi, Kuszynski, Bagchi, and Bagchi (2000) reported that treatment of human Chang liver cells with GSPE at a dose of 25 μg/ml reduced apoptosis by reducing the expression of a proapoptosis gene p53 and increasing the expression of cellular Bcl-2 gene. Furthermore, oral administration of GSPE (100 mg/kg) for 2 months to male rats against the risks induced by gibberellic acid has been found to ameliorate the levels of total lipids, total cholesterol, triglycerides, LDL-C, HDL-C, AST, ALT, urea and creatinine in serum. These observations confirmed the heptonephro-protective effect of GSPE against gibberellic acid induced oxidative stress and cellular alterations in rats (Hassan & Al-Rawi, 2013).

Uma Maheswari and Rao (2005) investigated that oral administration of grape seed oil (3.7 g/kg) for 7 days resulted in a significant decrease in ALP, AST, ALT, liver MDA and hydroperoxides levels and significant improvement in GSH, Superoxide dismutase, catalase, and total protein, when compared with CCl_4 damaged rats. An ex vivo study has shown that addition of GSP to a system containing polyunsaturated fatty acids and mice liver microsomes inhibited UV-C induced peroxidation of polyunsaturated fatty acid (Bouhamidi et al., 1998). In one of the studies, the beneficial effect of using GSE for three months in adult patients with nonalcoholic fatty liver disease has been investigated in comparison to treatment with vitamin C. The GSE was found to improve the grade of fatty liver change, and also resulted in decreased levels of ALT compared to those patients receiving vitamin C independently, from the initial grade of steatosis (Khoshbaten et al., 2010). In vivo studies done on animals have shown that administration of GS-supplemented food significantly protected the rats against alcohol-induced hepatotoxicity as evidenced by reduced levels of ALT, AST and LDH serum enzyme and MDA contents in all the tissues (Dogan & Celik, 2012). Similarly, the pre-treatment of liver slices with GSE has been found to inhibit the ethanol-induced oxidative stress in the liver by means of suppressing lipid peroxidation and protein carbonylation (Hassan, 2012). Last but not the least, GSP (10, 20, and 40 mg/kg/day for 3 weeks, intraperitoneally) showed protective effects against liver ischemia and reperfusion injury and reduced hepatic fat content particularly in HF- induced obese mice (Song et al., 2012). GSPE in combination with oil rich in docosahexaenoic acid has been found to modify the hepatic antioxidant enzymatic activities (Glutathione peroxidase GST and Glutathione-S-transferase GPx), which resulted in the prevention of the transient imbalance between the lipid hydroperoxide level and antioxidant status related to a lipidic postprandial state (Fernández-Iglesias et al., 2014). The ethanolic GSE administration to streptozotocin-induced male diabetic rats for 28 consecutive days helps to prevent liver damage due to oxidative stress which may contribute towards improvement in liver function and histology (Giribabu, Kumar, Rekha, Muniandy, & Salleh, 2014). Combined administration of GSE (200 mg/kg) with silymarin (50 mg/kg) effectively attenuate thioacetamide (TAA)-induced hepatic fibrosis in Sprague–Dawley rats and significantly improved the tested biochemical parameters, decreased hepatic hydroxyproline content, prevent the oxidative stress and restoring GSH level in experimental hepatic fibrosis (Nada et al., 2015).

5.6. Antimicrobial activity

GSEs have been shown to have strong antimicrobial activity. Palma and Taylor (1999) evaluated the antimicrobial activity of GSE using assorted microorganisms. During the experiment the authors found that the lipophilic fraction of GSE obtained with pure CO_2 was showing a high degree of antimicrobial activity at a concentration 1500 μg/4-mm disk, especially against human pathogenic microorganisms *Staphylococcus aureus* (+), *Staphylococcus coagulans niger* (+), *Citrobacter freundii* (−), *Escherichia cloacae* (−), *Escherichia coli* (−) and phytopathogenic fungi *Cladosporium echinulatum*. In another study, Ahn, Grun, and Mustapha (2004) demonstrated the inhibitory activity of a commercial GSE (ActiVin) against both Gram-negative bacteria, *Salmonella typhimurium* and *E. coli*, and Gram-positive bacteria *Listeria monocytogenes*, with *L. monocytogenes* being more sensitive to the inhibitory effect of GSE than the other two bacterial species assayed. Furthermore, the lowest inhibitory concentration of GSE which caused the complete inhibition of all three pathogens after 6 days of exposure was found to be ≥6 mg/ml. A study done by Shoko et al. (1999) have reported the antimicrobial activity of methanolic extract from grape seeds. They also confirmed that phenolics especially gallic acid was the most important compound active against *E. coli* and *Salmonella enteritidis*. Structural activity of correlation assays also revealed that the three hydroxyl groups of gallic acid derivates were effective for antibacterial activity. Recently, Rhodes, Mitchell, Wilson, and Melton (2006) reported antilisterial activity of a GSE of *Vitis vinifera* var. Ribier black table grapes. They reported that polymeric phenolic fractions exhibited the highest inhibition activity for all Listerial species tested, but not for other bacteria, such as *Bacillus cereus*, *Salmonella* Menston, *E. coli*, *S. aureus* or *Yersinia enterocolitica*. The GSE was found to decrease the *L. monocytogenes* numbers from 10^6–10^7 CFU/ml to no detectable colonies within 10 min. Similarly, Theivendran, Hettiarachchy, and Johnson (2006) have also reported the antimicrobial activity of GSE, both alone as well as in combination with nisin, against *L. monocytogenes*.

Jayaprakasha et al. (2003) have investigated the antimicrobial activity of two fractions of GSE against Gram-positive bacteria *B. cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *S. aureus* and the Gram-negative bacteria *E. coli* and *Pseudomonas aeruginosa*. The authors observed that the fraction containing higher degree of oligomers had greater antimicrobial activity compared to the fraction containing lower levels of these oligomers. The minimum inhibitory concentration levels of GSEs have been found to be 850–1000 ppm for Gram-positive bacteria and 1250–1500 ppm for Gram-negative bacteria. By using the paper disk diffusion method against some food spoilage and pathogenic bacteria such as *Aeromonas hydrophila* ATCC 7965, *Bacillus amyloliquefaciens* ATCC 3842, *Bacillus brevis* FMC 3, *B. cereus* FMC 19, *Bacillus megaterium* DSM 32, *B. subtilis* IMG 22, *Enterobacter aerogenes* CCM 2531, *Enterococcus feacalis* ATCC 15753, *E. coli* DM, *Klebsiella pneumonia* FMC 5, *L. monocytogenes* ScottA, *Mycobacterium smegmatis* RUT, *Proteus vulgaris* FMC 1, *P. aeruginosa* ATCC 27853 and *S. aureus* COWAN 1, the GSE at 20% concentration has been found to exhibit antibacterial effects against all these bacteria except *B. amyloliquefaciens*. The authors also determined that acetone:water:acetic acid as a solvent was more effective than its methanol counterpart (Baydar, Ozkan, & Sagdic, 2004). Furthermore, the researchers have reported the antibacterial effects of three GSEs obtained from three different varieties of *V. vinifera* against several bacteria, including *S. aureus*, *E. faecalis*, *E. coli*, and *S. enteritidis*, at different concentrations of 0.5%, 1%, 2.5% and 5%. They also found that these extracts contained high content of total phenolic compounds (Baydar, Sagdic, Ozkan, & Cetin, 2006).

The antibacterial activity of a GSE has also been investigated against different species and strains of Campylobacter. Growth inhibition was seen in the range from 5.08 to 6.97 log CFU/ml thereby demonstrating the strong efficacy of the GSE to inhibit Campylobacter growth (Silván et al., 2013). Moreover, a recent study has evaluated the potential antimicrobial use of GSE to inhibit the growth of *Alicyclobacillus acidoterrestris* cells and spores in apple juice during storage at 37 °C (Molva & Baysal, 2015).

5.7. Neuroprotective and anti-aging activity

The presence and accumulation of amyloid-β peptides play a key role in the development of Alzheimer's disease. Recently, it has been observed that administration of grape seed polyphenolic extract at a dose of 200 mg/kg/day orally for 5 months to Tg2576 mice attenuated Alzheimer's disease type cognitive deterioration with reduced amounts of high molecular weight amyloid β-protein in the brain (Wang et al., 2008). Moreover, oral administration of GSPE inhibited the development of tau-mediated neuropathology in the TMHT mouse model of tauopathy (Wang et al., 2010a; Wang et al., 2010b). Researchers have also for the first time observed that GSPE is capable of disrupting and disintegrating the filamentous structure of paired helical filaments found in Alzheimer's disease brain. The structural modifications seen associated with GSPE include end splaying, widening of filaments and disintegration of paired helical filaments into smaller fragments. These findings indicate that GSPE represent a potential therapeutic agent for treating Alzheimer's disease and other tau-mediated neurodegenerative conditions (Ksiezak-Reding et al., 2012). By using proteomics technology, Kim, Deshane, Barnes, and Meleth (2006) have demonstrated that GSE supplement accentuate the actions of psychoactive drugs in adult rat brain by way of affecting the proteins implicated in cognitive disorders. Mahmoud (2013) have reported the ability of GSE at a dose of 150 mg/kg body weight to antagonize the neurotoxic activity of *Cerastes cerastes cerastes* post-synaptic neurotoxin in sublethally intoxicated mice, either before or after intoxication. GSPE supplementation administered to adult rats resulted in possible alteration in the cholinergic system by way of increase in the acetylcholine concentration with a moderate reduction in acetylcholine esterase activity, further suggesting that GSPE has a beneficial role in minimizing age-related loss of cognition in older rats (Devi, Jolitha, & Ishii, 2006).

Asha, Sagar, Manjula, and Ishii (2011) have observed that GSPE reduced oxidative stress-related lipofuscin accumulation with age in the hippocampus of rats. Therefore, these results indicate that GSPE has a neuroprotectant role in the hippocampus and in preventing cognitive loss with aging. Balu, Sangeetha, Murali, and Panneerselvam (2005) have reported that GSE supplementation to aged rats at a dose of 100 mg/kg body weight for 30 days increased memory performance, decreased ROS production and inhibited the accumulation of age-related oxidative DNA damage in the spinal cord and other brain regions. Furthermore, administration of GSE at a dose of 100 mg/kg by oral gavage for 30 days to aged rats shows remarkable cognitive-enhancing activity in Morris water maze tests (Sarkaki, Farbood, & Badavi, 2007). In addition, Hwang et al. (2004) have demonstrated that oral administration of GSE at a dose of 60 mg/kg 30 min before or after experimental forebrain ischemia prevented gerbil hippocampal neurons from oxidative DNA damage. More recently, researchers have found the differential neuroprotective potential from two different GSE varieties, attributed to the low molecular weight polyphenols. Out of these two GSE varieties, Koshu, a white, local variety of *V. vinifera* protected cultured hippocampal neurons against glutamate-induced insults of acute inactivation of Erk1/2 and dendrite retraction (Narita, Hisamoto, Okuda, & Takeda, 2011). In another study, the cytoprotective effects of GSPE at a concentration of 100 and 300 mg/ml has been found to prevent the neurotoxicity induced by 6-hydroxydopamine in a cell-based study (Wu, Liao, Hsu, et al., 2010).

5.8. Skin protective activities

Voluntary Cosmetic Registration Program data obtained from the Food and Drug Administration in 2012 has indicated that GSE is used in 495 cosmetic formulations (Food and Drug Administration (FDA), 2012). In a placebo controlled study, dietary supplement of GSE alongside with Vitamin C, Vitamin E, tomato extract, Soy isoflavones and fish polysaccharides has been found to improve the condition, firmness and structure of the skin in healthy post-menopausal females (Skovgaard, Jensen, & Sigler, 2006). In vitro study have shown that pretreatment of cells with GSPE containing 5000 ppm of trans-resveratrol prominently upregulate oxidant and tumor necrosis factor alpha inducible vascular endothelial growth factor expression in human Ha-CaT keratinocytes (Khanna, Roy, Bagchi, Bagchi, & Ken, 2001). In addition to that, topical application of GSPE on dorsal skin wound in male mice accelerated wound contraction and closure by increasing the expression of vascular endothelial growth factor and tenascin. Therefore, GSPE containing resveratrol can be used to treat dermal wounds and other dermal disorders (Khanna et al., 2002). Studies by Yamakoshi et al. (2003) have also reported that GSE significantly suppresses melanin pigment formation as well as UV-induced hyperpigmentation in guinea pigs. The latter effect has been found to be stronger with GSE than with vitamin C. Similarly, in a one-year open design study on chloasma, researchers have demonstrated that oral administration of GSPE for 6 months effectively reduced the hyperpigmentation of women with chloasma and no further improvement was seen after this period (Yamakoshi, Sano, Tokutake, et al., 2004). More recently, it has been evaluated by Costa et al. (2012) that daily intake of a nutraceutical product containing GSE along with acerola extract, lycopene and Biomarine ComplexT (an extract rich in marine proteins and polysaccharides) showed prominent adjuvant effect to counteract skin photoaging, causes reduction of facial seborrhea, an increase of dermal density and maintain skin hydration, as assessed by ultrasound.

5.9. Anti-inflammation activity

It has been demonstrated that proanthocyanidins in GSEs have high anti-inflammatory action, because they scavenge free radicals, prevent lipid peroxidation and inhibit formation of pro-inflammatory cytokines (Georgiev et al., 2014). Li, Zhang, Wu, and Tian (2001) reported that GSPE could prevent the increase of MDA in rat paws with arthritis induced by carrageenan. Inflammatory cytokines like NO, interleukin-1β, tumor necrosis factor alpha and prostaglandin E_2 were successfully inhibited by proanthocyanidins. Another study demonstrated that GSP administered orally at a dose of 2 mg/kg three times daily for 6 days attenuated the carrageenan- or dextran-induced hind paw edema and prevents an increase in capillary permeability caused by local cutaneous application of xylene (Zafirov, Bredy-Dobreva, Litchev, & Papasova, 1990). Study done by Ma et al. (2007) showed that GSPE displayed anti-inflammatory activity, subsequently leading to decreased expression of high level vascular cell adhesion molecule-1 induced by AGEs through activation of peroxisome proliferators-activated receptor gamma expression and inhibition of receptor for AGE expression in human umbilical vein endothelial cells. In another study, it was found that GSP provide protection to endotoxin-stimulated RAW 264.7 macrophages from overproduction of inflammatory mediators, mainly NO and prostaglandin E_2 production, suppression of inducible nitric oxide synthase expression, and by inhibition of nuclear factor kB(p65) translocation to nucleus (Terra et al., 2007). In vivo studies have showed that HF-fed rats supplemented with grape seed procyanidins (7 mg/day for 19 weeks) attenuated inflammatory markers in liver, white adipose tissue, and circulation and are associated with the inhibition of the pro-inflammatory molecules C-reactive protein, interleukin-6 and tumor necrosis factor alpha and the enhanced production of the anti-inflammatory cytokine adiponectin (Terra et al., 2009). Similarly, one

more study has showed that orally ingested GSE helps in preventing the imbalanced cytokine patterns (Terra et al., 2011).

Carini, Stefani, Aldini, Orioli, and Maffei Facino (2001) demonstrated that GSP actively restrain the inflammatory response of activated neutrophils due to their membrane-stabilizing effect, thus preventing neutrophils adhesion and activation during an inflammatory response. It has been reported that GSPE dose-dependently suppressed the severity of collagen-induced arthritis, reduced oxidative stress, inflammatory cytokine production and serum levels of type-II-collagen-specific IgG2a in mice (Cho et al., 2009). Researchers have also found that GSE in a dose of 100 mg/day for a month attenuated the increased expression of adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin) as a result of inflammatory response in the plasma of systemic sclerosis patients (Kalin et al., 2002). In a recent study, Sano, Tokutake, and Seo (2013) have demonstrated the ability of GSE to reduce leg swelling sometimes seen with sitting for prolonged periods of time. It was observed that GSE at a dose of 400 mg taken for 2 weeks reduced the leg volume (via swelling) by 70%. Furthermore, GSPE exerts a beneficial anti-inflammatory effect in the recurrent phase of 2,4,6-Trinitrobenzene sulfonic acid-induced ulcerative colitis in rats. This anti-inflammatory activity exerted by the GSPE was through the increase of anti-inflammatory cytokines (IL-2 and IL-4) and a decrease in pro-inflammatory cytokines such as interleukin-1β in the colonic tissues, thereby, inhibiting inflammatory cell infiltration and anti-oxidative damage (Wang et al., 2010a; Wang et al., 2010b; Li, Cai, et al., 2008).

In a recent study, Zucker fa/fa rats treated with a dose of 35 mg/kg/day of GSPE for a period of 10 weeks exerted an anti-inflammatory effect in genetically obese rats by modulating the expression of several genes in the adipose tissue and being the adipocyte a target cell of procyanidins (Pallarès et al., 2013).

6. Grape seed extract & other diseases

Saito, Hosoyama, Ariga, Kataoka, and Yamaji (1998) have demonstrated that in rats with experimentally-induced gastric mucosal ulcers, prominent protection was seen by treatment with GSE at a dose of 200 mg/kg body weight. This protective activity of GSE has been found to be higher in high flavonol containing extracts than in those with a low flavonol content. The authors also observed that higher molecular weight flavan-3-ol oligomers (tetramers, pentamers, and hexamers) of GSEs showed antiulcer activity while as lower molecular weight flavan-3-ol (monomers, dimers and trimers) do not exhibit this activity. It has also been reported that oral administration of GSE at a dose of 25 and 250 mg/kg attenuated ethanol-induced ulcers more effectively than Vitamin C and Vitamin E, but its protection against aspirin-induced ulcers was comparable for all treatments, while on the other hand GSE reduced the gastric MDA levels elicited by these aggressive agents more significantly than Vitamin C, but similar to Vitamin E (Cuevas, Calzado, Guerra, Year, & Despaigne, 2011). Furthermore, in vivo animal experimental study has reported that rats pretreated with 100 mg GSPE/kg/day for 15 consecutive days decreased acute stress- and chronic stress-induced lipid peroxidation, DNA fragmentation and membrane microviscosity in the gastric mucosa and intestinal mucosa (Bagchi, Bagchi, Ray, Kuszynski, & Stohs, 1999; Bagchi et al., 1999). Similarly, another study done by Bagchi and Bagchi (2001) have demonstrated the protective ability of GSPE for reducing or preventing gastric injury induced by stress in an animal model.

In vivo experimental study has shown that hereditary cataractous rats (ICR/f rats) fed with a diet containing 0.213% of GSE for 27 days significantly prevented and postponed the progression of cataract formation mainly because of their antioxidant activity (Yamakoshi, Saito, Kataoka, & Tokutake, 2002a). In a similar study consumption of GSPE in a dose of 250 mg/ml in rats offer a prophylactic effect against selenite-induced oxidative cataract, causing slowing or inhibition of cataract formation (Durukan et al., 2006). Furthermore, GSPE has been reported to play an important role in protecting human lens epithelial B-3 (HLEB-3) cells against H_2O_2-induced oxidative stress and p38 and JNK signaling pathway. Hence, suggesting that GSPE has a significant protective activity against cataractogenesis (Jia, Song, Zhao, Wang, & Liu, 2011).

In a double blind, placebo controlled, parallel arm study which lasted for 8 weeks was undertaken to determine whether a GSE lowers blood pressure in subjects with prehypertension. It was found that GSE at a dose of 300 mg/day significantly lowers both the systolic and diastolic blood pressures (Robinson, Lu, Edirisinghe, & Kappagoda, 2012). In humans, the GSE was also found to lower blood pressure in patients diagnosed with the metabolic syndrome (Sivaprakasapillai et al., 2009). By using centrifugal partition chromatography method a galloylated procyanidin dimer has been isolated from GSPs which showed promising results in the dentin stiffness bioassay and indicates its potential role as dentin biomodifiers to be used in restorative and reparative dentistry (Phansalkar et al., 2015). In vitro studies have also showed that GSEs, which contain high amount of proanthocyanidins, positively affected the demineralization and/or remineralization processes of artificial root caries lesions, suggesting their use as promising natural agents for non-invasive root caries prevention (Lorenzo et al., 2015).

One of the studies has demonstrated the effect of commercial GSE on human enteric virus surrogates and hepatitis A virus. During the study, researchers evaluated the infectivity of the virus exposed to GSE concentrations of 0.5, 1, and 2 mg/ml for 2 h at room temperature or 37 °C, suggesting that GSE has the potential to be used by the food industry as an inexpensive novel natural broad-spectrum antiviral alternative to reduce viral contamination and enhance food safety and quality (Friedman, 2014). Nair et al. (2002) have found that the flavonoid constituents of GSE exert antiviral effects by inducing Th1-derived cytokine γ-interferon (IFN-γ) by peripheral mononuclear cells, suggesting that the beneficial immunostimulatory effect of GSE may be mediated through induction of IFN-γ (Nair et al., 2002; Bak, Truong, Kang, Jun, & Jeong, 2013). The amount of polyphenols in GSE is also correlated with increased antiemetic effect (Olaku et al., 2015).

7. Toxicological studies

A series of toxicological studies have been performed to investigate the safety profile of GSEs containing proanthocyanidin. Yamakoshi, Saito, Kotaoka, and Kikuchi (2002b) have assessed the safety of GSE in Fischer 344 rats and observed that the no-observed-adverse-effect level in their subchronic toxicity study was 2% of the diet, which correlates to a dietary intake of 1.4 and 1.5 g/kg body weight/day in males and females, respectively. In acute oral toxicity studies in rats, GSE has been found to be well tolerated and safe with an LD50 of 5000 mg/kg with single oral dose via gastric intubation. Furthermore, a 6 months chronic toxicity studies done on female B6C3F1 mice has shown that administration of GSE at a dose of 100, 250 or 500 mg/kg/day is safe with no treatment-related mortality and no significant changes in body weight or physical appearance (Ray, Bagchi, Lim, et al., 2001). In another 90-day oral toxicity studies, administration of water extracted GSE that contained less than 5.5% of catechin monomers to 4 groups of rats (20 rats/sex/group) at levels of 0, 0.5, 1 .0, or 2.0% did not show any clinical signs of toxicity or reduction in bodyweight (Wren, Cleary, Frantz, Melton, & Leslie, 2002). Bentivegna and Whitney (2002) have also reported that a diet containing 2.5% of GSE showed no-observed-adverse effect level with no ophthalmic changes and no histological findings that indicate toxicity in the rats, hence supporting the safety and tolerability of GSE in experimental animal models. Furthermore, in a litmus test using female Wistar rats acute oral toxicity study of GSE has been determined. The LD50 has been reported to be higher than 5000 mg/kg when GSE was administered orally via gastric intubation. Moreover, there has been no observed toxicity effects in rats for a period of 14 days after dosing, and no gross lesions were observed at necropsy (Lluis, Munoz, Nogues, et al., 2011). Similarly, the

administration of GSE at a dose of 100 mg/kg body weight by gavage for 7 to 10 days to adult male ICR (CD-I) mice have been found to provide multiorgan protection against drug- and chemical-induced toxicity with no significant changes in serum creatine kinase and ALT activity, blood urea nitrogen level, DNA fragmentation, or organ histopathology compared to the saline control groups (Bagchi, Ray, Patel, & Bagchi, 2001). In addition, the LD50 of GSPE has been found to be higher than 2000 mg/kg body weight when administered once dermally for 24 h to the clipped, intact skin of five male and five female albino rats. During the study no deaths, body weight changes, or gross necropsy has been observed (Bagchi, Bagchi, Stohs, et al., 2000).

8. Conclusion

Today, the advent of evidence based practices, experimental support for plant-derived preparations and molecules has gained acceptance and validity, thus enhancing their translational effectiveness in clinics. GSEs containing proanthocyanidins are positioned as strong antioxidants and free radical scavengers. In recent times, the evidence is emerging on the potential benefits and lack of toxicity or mutational propensity discovered through research, that use of these extracts in animal and human clinical studies can be supported. Therefore, the findings have suggested that grape seeds and their active constituents should be studied in a more detailed manner for development as therapeutic agents to assist in the attenuation of cardiovascular, gastrointestinal, and neurodegenerative diseases. Moreover, potential use of these natural extracts could offer the food processing industries an alternative solution to synthetic chemical antimicrobials and antioxidants. At the same time, identification and purification of these bioactive constituents from GSE would be essential to optimize formulations of these extracts for medical or nutritional purposes. Furthermore, mechanistic and clinical studies are required to unravel the mechanism of action of GSEs.

Acknowledgment

Declaration of interest. The authors declare no conflict of interest.

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