Pharmacognosy Reviews [Phcog Rev.]
Vol 2, Issue 4, Jul-Dec, 2008
Page 303-308

PHCOG REV.
An official Publication of Phcog.Net

PHCOG REV.: Review Article

Anti-aging Activities associated with Grape Seeds -A Review

Mustafa G*[a]., Ansari S.H.[a], Imtiyaz M.[a], Bhat Z.A.[b]

[a]*Herbal Cosmetics & Immunomodulatory Lab, Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar New Delhi -110062, India.*

[b] *Depatment of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar, J & K- 190006, India*

**Address for correspondence to: Gh.Mustafa Khan, Herbal Cosmetics & Immunomodulatory Lab, Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar New Delhi -110062, India.*

Email: khanmustafa111@gmail.com/khan_mustafa111@yahoo.co.in

ABSTRACT

Radical scavengers have attracted special interest because they can protect the human body from free radicals that may cause many diseases including cancer and lead to the aging process. The compounds isolated from grape seeds have found to be efficient free radical scavengers even at the weak concentrations. This review is written keeping in view the potential benefits of grape seeds in a wide variety of ailments and to describe their anti – aging properties.

Key words: GSPE, Grape Seed Proanthocyanidin Extract; GSE, Grape Seed Extract;

INTRODUCTION

Aging is a very complex biological process. In addition to individual genetic factors also external influences, such as nutrition, smoking, alcohol, environmental conditions, etc. can strongly contribute to its anticipated appearance. A particular attention in this respect has been paid to the biological action of free radicals, especially to oxygen species (OH, peroxyl, ozone and other oxidizing species), which are causing "oxidative stress" (1). It appears that these transients in addition to the nitrogen oxides are one of the major factors for a forced aging, DNA- damage, carcinogenesis and for initiation of a number of diseases. Aging comprises various changes that occur in living organisms with the passage of time, leading to increased system entropy, loss of homeostasis and eventually death. Of the various theories proposed, the free radical theory of aging states that it is the shift in antioxidant/pro-oxidant balance that leads to increased oxidative stress, dysregulation of cellular function and aging. It has been proved that accumulated damage by free radicals produced by exposure to ultraviolet (UV) radiation results in extensive damage to the soft skin tissues, which is commonly known as "photo aging". Although the human body has built-in antioxidant mechanisms to suppress uncontrolled free radicals, these mechanisms may at times fail due to overwhelming production of toxic radicals. This is where supplemental antioxidants can play a vital role in restoring the balance, lack of which produces visible wrinkling and premature aging (2).

The role antioxidants have in free radical stabilization involves the antioxidants donating one of their own electrons to the free radical. This electron donation is done without the antioxidant becoming unstable or damaging to the body. This remarkable action stabilizes the free radicals as quickly as they are produced in the human body.

The antioxidants may be enzymatic or non enzymatic, superoxide dismuatse, glutathione peroxidase, catalase and peroxidases are some examples which come under enzymatically potential antioxidants. In the non-enzymatic category, some of the known antioxidants are Vitamin C, Vitamin E, Vitamin A, beta-Carotenenoids, Uric acid, Ubiquinone and synthetic compounds like Butylhydroxy toluene (BHT), Butylhydroxyanisole (BHA), melatonin, Dihydro-epiandrosterone (DHEA) etc (3).

Antioxidants using natural sources: Antioxidants of natural origin have attracted special interest because they can protect human body from free radicals without producing toxic effects (4). It is already reported that natural antioxidants, especially phenolics and flavonoids, found in plants are the most bioactive (5). Plants available worldwide already reported for their antioxidant activity are well known, famous for their uses and readily available.

Grape seeds (*Vitis vinifera* L.)

Kingdom	*Plantae* - Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* - Monocotyledons
Order	*Vitales*
Family	*Vitaceae*
Genus	*Vitis*
Species	*Vitis vinifera*
Synonyms	
HINDI-	*Angur, dakh*
BENG.-	*Angurphal, drakhyaluta*
MAR.-	*Draksha*;
GUJ.-	*Darakh, draksha*;
TEL.-	*Draksha, gostanidraksha;*
TAM.-	*Kodimundri, gostanidraksha*;
KAN.-	*Angura, draksha*
KASH.-	*Dach*

A large deciduous climber, tendrils long bifid. Leaves 7.5-15 cm long, more or less deeply 3-5 lobed, margin irregularly and coarsely toothed. Flowers green. Berry very variable in size, bluish black or greenish. Seeds, pear shaped, with a discoidal tubercle on the back from which a low ridge runs over the top

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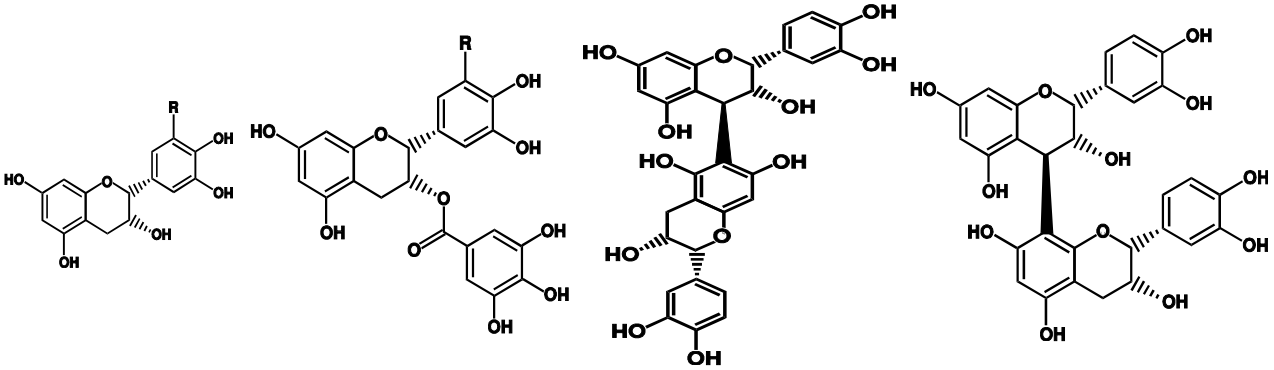
and down the ventral face. A native of W. Asia and cultivated in many parts of india especially in N.W. India (6).

Chemical Composition: The chemistry of the grape is complex and has been studied in considerable detail. White grapes have a straw-yellow colour due to flavonone compounds such as quercetin and quercitrin. Red and black grapes, on the other hand, contain pigments of the anthocyanin type, in particular malvidin 3-glucoside. Both leaves and fruit of the grape vine contain a variety of other flavonoid compounds, including (+)-catechin, (-)-epicatechin, (+/-)- gallocatechin, (-)-epicatechin 3-O-gallate, rutin, and luteolin (**7**). Grape skins contain the polyphenolic defence compound resveratrol which has attracted attention for its anticancer activity and its serum lipid-lowering and antiplatelet effects (8,9). Standardized grape seed extracts are reported to contain 92-95% oligomeric proanthocyanidins (OPCs) (10). Although the chemical composition has not been elucidated completely, the main constituents of grape seeds are phenolic compounds, broadly divided into monomers (catechin, epicatechin) and condensed flavonoids of various chain lengths. Grape seeds, in particular the slimy film that surrounds the seed, are rich in polyphenolic compounds known as procyanidins. Procyanidins are a class of proanthocyanidins (condensed tannins) consisting of oligomers of catechin and epicatechin units. Procyanidins are characteristic of the grape seed, whereas other proanthocyanidins occur in other parts of the fruit (11).

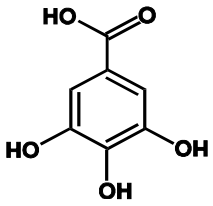
Main grape seed constituents:

- (-)-epicatechin gallate, procyanidin dimers, trimers, tetramers, and their gallates 80%;
- (+)-catechin, (-)-epicatechin, and gallic acid 15%;
- procyanidin pentamers, hexamers, and heptamers, and their gallates 5%.

In addition to the above mentioned compounds grape seeds contain lipid, protein and carbohydrate (12).



R=H catechin and R=OH, gallocatechin | *Epicatechin-3-O-gallate* | *4-6 linked epicatechin* | *4-8 linked epicatechin*



Gallic acid

Fig. 2.2. Chemical constituents of grape seed

Anti – aging activities

Antioxidant activity: Scientific studies have shown that the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C. Extensive research suggests that grape seed extract is beneficial in many areas of health because of its antioxidant effect to bond with collagen, promoting youthful skin, cell health, elasticity, and flexibility. Other studies have shown that proanthocyanidins help to protect the body from sun damage, to improve vision, to improve flexibility in joints, arteries, and body tissues such as the heart, and to improve blood circulation by strengthening capillaries, arteries, and veins (12). The flavonoid constituents of grape seed extract have antioxidant and free radical scavenging activity. Procyanidins from grape seed scavenge the superoxide radical. Grape seed procyanidins also trap hydroxyl radicals and prevent both iron-promoted and ultrasound-induced lipid peroxidation *in vitro*; they are far more active than vitamin E in these respects (13).

Inhibition of lipid peroxidation: The antioxidant activity of procyanidins from grape *(Vitis vinifera)* seeds has been extensively studied since the finding of the French Paradox. The antioxidant properties of procyanidins from grape seeds have been shown to inhibit superoxide anion and lipid peroxidation and to reduce or delay the formation of conjugated dienes during all phases of lipid peroxidation including induction, propagation and breakdown (14). GSE has shown to enhance the antioxidant status and decreased the

incidence of free radical-induced lipid peroxidation in blood samples of rats exposed to x-radiation. The antioxidant effect of GSE given to animals has proved more effective than vitamin E administered before whole-body irradiation in rats (15). Following consecutive 12-wk administration of tablets containing 0, 200 or 400 mg grape seed extract (calculated as proanthocyanidin) to 61 healthy subjects with LDL cholesterol (LDL-C) levels of 100 to 180 mg/dL, effects of such treatment compared to administration of placebo tablets on malondialdehyde-modified LDL (MDA-LDL), representing one oxidized type of LDL, were investigated by a single blind method. MDA-LDL level in the 200 mg (calculated as proanthocyanidin) group was significantly ($p < 0.05$) reduced compared to the basal level, 12 wk after the start of administration. These results suggested that tablets containing grape seed extract exerted reducing effects on oxidized LDL, and might be useful in preventing lifestyle-related diseases such as arteriosclerosis (16).

Hepatoprotective activity:

To assess the protective effect of grape seed extract (GSE) against oxidative liver injury and fibrosis induced by biliary obstruction in rats, wistar albino rats were divided into four groups; control (C), GSE-treated, bile duct ligated (BDL), and BDL and GSE-treated (BDL + GSE) groups. GSE was administered at a dose of 50 mg/kg a day orally for 28 days. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels were determined to assess liver function and tissue damage, respectively. Tumor necrosis factor-alpha (TNF-alpha) and antioxidant capacity (AOC) were assayed in plasma samples. Liver tissues were taken for determination of the hepatic malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and collagen content. Production of reactive oxidants was monitored by chemiluminescence (CL) assay. Results: Serum AST, ALT, LDH and plasma TNF-alpha were elevated in the BDL group as compared to the control group and were significantly decreased with GSE treatment. Plasma AOC and hepatic GSH level, depressed by BDL, was elevated back to the control level in the GSE-treated BDL group. Increases in tissue MDA level, MPO activity and collagen content due to BDL were also attenuated by GSE treatment. Furthermore, luminol and lucigenin CL values in the BDL group increased dramatically compared to the control and were reduced by GSE treatment. These results have suggested that GSE protects the liver from oxidative damage following bile duct ligation in rats and this effect possibly involves the inhibition of neutrophil infiltration and lipid peroxidation; thus, restoration of oxidant and antioxidant status in the tissue (17).

Defatted milled grape seed (DMGS) a wine by-product obtained from the oil extraction of the grape seed that contains different types of phenolic compounds. A study was designed to evaluate the possible protective effect of DMGS on toxicity induced by adriamycin (ADR) in isolated rat hepatocytes. The study was carried out by examining the results of lactate dehydrogenase (LDH) release to estimate cytotoxicity; the thiobarbituric acid reactant substances (TBARS) and carbonyl group levels were measured as biomarkers of oxidative stress and ATP and GSH levels as estimation of intracellular effect. The results showed that DMGS extract protects the cellular membrane from oxidative damage and consequently prevents protein and lipid oxidation. The levels of ATP and GSH changes for the ADR toxicity were restored to control value in the presence of DMGS extract. The experimental results suggest that this wine by-product may be used to decrease oxidative stress (18).

Neuroprotective activity: Aging the accumulation of diverse deleterious changes in the cells and tissues leading to increased risk of diseases. Oxidative stress is considered as a major risk factor and contributes to age related increase in DNA oxidation and DNA protein cross-links in central nervous system during aging. The salubrious role of grape seed extract was evaluated on accumulation of oxidative DNA damage products such as 8-OHdG and DNA protein cross-links in aged rats. Male albino rats of Wistar strain were divided into four groups: Group I, young control rats; Group II, young rats treated with grape seed extract (100mg/kgb.wt.) for 30 days; Group III, aged control rats; Group IV, aged rats supplemented with grape seed extract (100mg/kgb.wt.) for 30 days. Results, have revealed that grape seed extract has inhibiting effect on the accumulation of age-related oxidative DNA damages in spinal cord and in various brain regions such as cerebral cortex, striatum and hippocampus (19).

Grape seed extract improved the histopathologic brain score in cortex, hippocampus and thalamus ($P<0.05$ versus vehicle). Concentrations of brain 8-isoprostaglandin F2alpha and thiobarbituric acid reacting substances significantly increased due to hypoxic ischemia. Grape seed extract reduced this increase. Treatment with grape seed extract suppresses lipid peroxidation and reduces hypoxic ischemic brain injury in neonatal rat (20).

Proteomics technology, namely 2D gel electrophoresis and mass spectrometry, identified quantitative changes in specific proteins induced in adult rat brain following ingestion of a powdered preparation of GSE conclusion is that GSE has neuroprotective activity, by affecting specific proteins in particular ways (21).

Renal protection: GSPE has the effect in protecting kidney of diabetic rats, the mechanism might be related with its action in increasing the renal antioxidative ability,decreasing the content of NO and the activity of NOS in kidney and serum (22). GSPE has insulinomimetic properties and activates glycogen and lipid synthesis. However, the differences between the effects of GSPE and the effects of insulin indicate that GSPE uses mechanisms complementary to those of insulin signaling pathways to bring about these effects (23).

Adaptogenic & Nootropic (Antistress) activity:

The seed extract of *V. vinifera* was evaluated for antistress activity in normal and stress induced rats. Furthermore, the extract was studied for nootropic activity in rats and in-vitro antioxidant potential to correlate its antistress activity. For the evaluation of antistress activity, groups of rats (n = 6) were subjected to forced swim stress one hour after daily treatment of *V. vinifera* extract. Urinary vanillylmandelic

acid (VMA) and ascorbic acid were selected as non-invasive biomarkers to assess the antistress activity. The 24 h urinary excretion of vanillylmandelic acid (VMA) and ascorbic acid were determined by spectrophotometric methods in all groups under normal and stressed conditions. The nootropic activity of the extract as determined from acquisition, retention and retrieval in rats was studied by conditioned avoidance response using Cook's pole climbing apparatus.

The in vitro antioxidant activity was determined based on the ability of *V. vinifera* to scavenge hydroxyl radicals. Daily administration of *V.vinifera* at doses of 100, 200 and 300 mg/kg body weight one hour prior to induction of stress inhibited the stress induced urinary biochemical changes in a dose dependent manner. However, no change in the urinary excretion of VMA and ascorbic acid was observed in normal animals at all the doses studied. The cognition, as determined by the acquisition, retention and recovery in rats was observed to be dose dependent. The extract also produced significant inhibition of hydroxyl radicals in comparison to ascorbic acid in a dose dependent manner. This study provides scientific support for the antistress (adaptogenic), antioxidant and nootropic activities of *V. vinifera* seed extract and substantiate the traditional claims for the usage of grape fruits and seeds in stress induced disorders (24).

Obesity & Weight control:

Since grape-seed extract has been shown to stimulate lipolysis in vitro and reduce food intake in rats, assessment of the efficacy of grape-seed extract with respect to energy intake (EI) and satiety was carried out. Grape seed reduced 24 h EI, with on average 4% in subjects who had an energy requirement > or =7.5 MJ/day, without further effects on satiety, mood or tolerance. These findings suggest that grape seed could be effective in reducing 24 h EI in normal to overweight dietary unrestrained subjects, and could, therefore, play a significant role in body-weight management (25).

The effects of grape seed extract (GSE) on the fat-metabolizing enzymes pancreatic lipase, lipoprotein lipase, and hormone-sensitive lipase in vitro was assessed and its potential application as a treatment for obesity evaluated. Crushed grape seeds were extracted in ethanol, and the extract was assayed for the measurement of inhibitory effects on pancreatic lipase and lipoprotein lipase activities and on lipolysis of 3T3-L1 adipocytes. The GSE rich in bioactive phytochemicals showed inhibitory activity on the fat-metabolizing enzymes pancreatic lipase and lipoprotein lipase, thus suggesting that GSE might be useful as a treatment to limit dietary fat absorption and the accumulation of fat in adipose tissue. The observed reduction in intracellular lipolytic activity of cultured 3T3-L1 adipocytes may reduce the levels of circulating free fatty acids that have been linked to insulin resistance in obese patients. The GSE rich in compounds that inhibit lipases may provide a safe, natural, and cost-effective weight control treatment (26).

Attenuation of drug & chemical toxins:

Comparative protective abilities of GSPE, and vitamins C and E, singly and in combination, were assessed against smokeless tobacco extract (STE)-induced oxidative stress, DNA fragmentation and apoptotic cell death in a primary culture of normal human oral keratinocytes. GSPE protected against STE-induced oxidative stress, DNA damage and apoptotic cell death, and provided better protection as compared to vitamins C and E, singly and in combination. The bioavailability and protective ability of GSPE were examined against acetaminophen (AP)-induced hepato- and nephrotoxicity, amiodarone (AM)-induced lung toxicity, doxorubicin (DX)-induced cardiotoxicity and dimethylnitrosamine (DM)-induced spleenotoxicity in mice. GSPE-fed animals were compared with GSPE-untreated mice to evaluate the protective ability of GSPE against these structurally diverse drugs/chemicals. Serum chemistry changes histopathology and DNA damage were evaluated. Results indicate that GSPE preexposure prior to the drugs/chemicals such as AP, AM, DX or DM treatment, provided near complete protection in terms of serum chemistry changes and inhibition of both forms of cell death, e.g., apoptosis and necrosis. DNA damage in various tissues triggered by these agents was significantly reduced in GSPE-fed animals. Histopathological examination of multiple target organs provided similar data. The results suggested that GSPE exposure is bioavailable and provides significant multiorgan protection against structurally diverse drug- and chemical-induced toxic assaults. Further, these studies exhibited a series of mechanistic information including free radical scavenging ability, anti-endonucleolytic activity, cytochrome P450 2E1 inhibitory activity, anti-necrotic, anti-apoptotic and anti-carcinogenic activities, modulatory effects on antioxidative and apoptotic regulatory genes such as Bcl2, c-myc and p53, which may be responsible for the novel chemoprotective properties exhibited by GSPE (27).

Free radical scavenging:

The concentration- or dose-dependent free radical scavenging ability of a novel IH636 grape seed proanthocyanidin extract (GSPE) both in vitro and in vivo models was assessed, and compared the free radical scavenging ability of GSPE with vitamins C, E and beta-carotene. These experiments demonstrated that GSPE is highly bioavailable and provides significantly greater protection against free radicals and free radical-induced lipid peroxidation and DNA damage than vitamins C, E and beta-carotene. GSPE was also shown to demonstrate cytotoxicity towards human breast, lung and gastric adenocarcinoma cells, while enhancing the growth and viability of normal human gastric mucosal cells. The comparative protective effects of GSPE, vitamins C and E were examined on tobacco-induced oxidative stress and apoptotic cell death in human oral keratinocytes. Oxidative tissue damage was determined by lipid peroxidation and DNA fragmentation, while apoptotic cell death was assessed by flow cytometry. GSPE provided significantly better protection as compared to vitamins C and E, singly and in combination. GSPE also demonstrated excellent protection against acetaminophen overdose-induced liver and kidney damage by regulating bcl-X(L) gene, DNA damage and presumably by reducing oxidative stress. GSPE demonstrated excellent

protection against myocardial ischemia-reperfusion injury and myocardial infarction in rats. GSPE was also shown to upregulate bcl(2) gene and downregulate the oncogene c-myc. Topical application of GSPE enhances sun protection factor in human volunteers, as well as supplementation of GSPE ameliorates chronic pancreatitis in humans. These results demonstrate that GSPE provides excellent protection against oxidative stress and free radical-mediated tissue injury (28).

Several enzymes involved in the degradation of structural components of the extravascular matrix are inhibited by grape seed procyanidins. This applies to some proteolytic enzymes such as collagenase and elastase and to some glycosidases such as hyaluronidase and beta-glucoronidase. This inhibition of enzyme activity may in part be due to trapping of reactive oxygen species by the procyanidins. Hence grape seed procyanidins may prevent oxidative injuries to the vascular endothelium (29).

Mechanism of action:

The beneficial effects of grape seed proanthocyanidins (GSPE) have been reported, however, little is known about their mechanism(s) of action. One of the beneficial effects of GSPE is chemoprevention of cellular damage. The precise mechanism by which GSPE mediates, chemoprevention is not yet understood. Mechanisms of actions of GSPE has been investigated by a study in which chemotherapy-induced toxic effects of Idarubicin (Ida) and 4,-hydroxyperoxycyclophosphamide (4-HC) were ameliorated in normal human Chang liver cells. Exposure to GSPE resulted in a significant reduction in apoptosis in response to the cytotoxicity of chemotherapeutic agents. RT-PCR analysis has shown a significant increase in the anti-apoptotic gene Bcl-2 and a decrease in the cell cycle associated and proapoptotic genes, c-myc and p53 in cells treated with GSPE. These results have suggested that some of the chemopreventive effects of GSPE are mediated by upregulating Bcl-2 and down regulating c-myc and p53 genes (30).

Bioavailability:

Absorption studies conducted with Caco-2 cells have revealed that dimers and trimers of proanthocyanidins are readily transported across the cell monolayers whereas higher polymers, (MW 1740 Da) were absorbed onto the epithelial cells and permeability was greatly reduced (31) . Studies with humans have corroborated these results, showing that dimeric proanthocyanidins, but not higher polymers , were identified in plasma after consumption of proanthocyanidin rich grape seed extract (32).

Safety:

Toxicological studies on long term (90 days) oral administration of GSPE to rats established a no-observed-adverse-efect level (NOAEL) of 1.4 g kg $BW^{-1}day^{-1}$ for males and 1.5 g kg $BW^{-1}day^{-1}$ for females (33).

Conclusion:

In the ever increasing demand of natural products for alleviating diverse health problems, grape seed have proven to possess multiple health benefits coupled with their health compatibility. The different activities of the grape seed extracts can be ascribed to their different phenolic compositions. It is now becoming necessary to isolate individual compounds which have shown the desirable benefits and also to perform bioanalytical studies to confirm their bioavailability, To this end, we need to study the scavenging activity of grape seed phenolic compounds in vivo. Further studies are needed to identify and study suitable grape varieties in ever more geographical areas than previously has been the case. In this manner new highly effective compounds from grape seeds can be discovered most expetiously through a multidisciplinary collabrative research efforts in the future.

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