

Protective Effects of Red Grape Seed Extracts on DNA, Brain and Erythrocytes Against Oxidative Damage

Hazem M.M. Hassan

Department of Biochemistry,
Faculty of Agriculture, Cairo University, Giza, Egypt

Abstract: The aim of this study was to investigate the protective role of red grape seed extracts (GSEs) against oxidative damage on DNA, brain and erythrocytes *in vitro*. In this study, the protective effects of two GSEs (water and ethanol) on free radical mediated DNA-sugar damage, Fe²⁺-induced lipid peroxidation in brain and H₂O₂ induced hemolysis and lipid peroxidation of erythrocytes were determined. The results revealed that the presence of various concentrations of each grape seed extract in the reaction mixture prevented the free radical-mediated DNA-sugar damage in a dose dependently. Similarly, GSEs caused a significant decrease in Fe²⁺-induced lipid peroxidation in rat brain tissues in a dose dependent manner. Both GSEs at different concentrations suppressed H₂O₂-induced hemolysis of rat erythrocytes in a concentration-dependent manner. Pre-treatment of cells with GSEs, however, prevented the phospholipid oxidative alteration in a concentration-dependent manner. Noteworthy, the ethanol grape seed extract (EGSE) was more effective than water grape seed extract (WGSE) against oxidative damage of DNA, brain and erythrocytes. In conclusion, the results demonstrated that GSEs protected DNA, brain and erythrocytes against oxidative stress and it could be used as a valuable food supplement or a nutraceutical product.

Key words: Brain • DNA • Erythrocytes • *In vitro* • Oxidative damage • Red grape seeds

INTRODUCTION

Reactive oxygen species (ROS) such as hydrogen peroxide, organo peroxide, superoxide anion and hydroxyl radical are generated in biological systems by aerobic metabolism and also by exogenous sources such as drugs, ultraviolet light, ionizing radiation and pollution systems [1]. According to generally accepted mechanisms, major deleterious effects are caused by hydroxyl radical (OH) generated from H₂O₂ and by the superoxide (O₂^{•-}) species generated in the presence of redox active transition metals [2]. Many endogenous and exogenous defense mechanisms are available in living organisms to limit the levels of ROS and the damage caused by these radicals [3]. The defense mechanisms include antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and many non-enzymatic antioxidant compounds such as polyphenols, tocopherols, ascorbic acid, uric acid, glutathione and other thiol protein groups to protect the functional and structural integrity of the biological

molecules such as proteins, lipids and nucleic acids [4]. It has been reported that the unbalanced ROS production and antioxidant cell defenses have been associated in the physiological and pathological conditions such as aging, cancer, rheumatoid arthritis, atherosclerosis and neurodegenerative diseases [5].

Epidemiological studies have strongly suggested that diet rich in fruits, vegetables and cereals plays a crucial role in the prevention of chronic diseases such as cardiovascular diseases and certain types of cancer by quenching free radicals. The beneficial health effects from the consumption of diet rich in fruits and vegetables are mainly due to the presence of antioxidants such as polyphenols, carotenoids and anthocyanins [6,7]. It has been observed that DNA is also a major target of oxidative injury [8], being of particular importance in a large number of disorders such as cancer [9] and degenerative diseases including Alzheimer's disease, Parkinson's disease and Hodgkin's disease [10, 11]. For years researchers have known that free radicals can cause cell degeneration, especially in the brain. The brain

and nervous system are particularly vulnerable to oxidative stress due to limited antioxidant capacity [12,13]. Erythrocytes can serve as a relevant human cell model in the investigation of bioavailability and antioxidant protection by natural products against oxidative stress [14,15].

Grape is one of the most commonly consumed fruits in the world. It has various biological functions, due to its rich polyphenol ingredients, most of which are contained in its seeds (60-70%) and skin (30%). However, large quantities of grape seed wastes are produced annually by the food processing industry-wine, juice etc [16]. Grape seed extract (GSE) contains a number of polyphenols, including procyanidins and proanthocyanidins, which are powerful free radical scavengers. Grape seed proanthocyanidin extract especially red grape seed extract is a rich source of polyphenolic antioxidants, a naturally occurring family of oligomeric proanthocyanidins found in a wide range of fruit and vegetables. A number of studies have demonstrated the superior free radical scavenging ability of GSE as compared to vitamins C, E and β -carotene [17,18]. Polyphenols in grape seeds have also been reported to have a variety of biological activities, including antioxidant, antithrombotic, antitumor, antibacterial, antiviral, anti-inflammatory, antiallergic, protection against X-ray and ultraviolet rays, chemoprevention, vasodilatory actions and cardioprotective effects [17-21]. Such remarkable spectrum of biochemical and cellular functions holds promise for the prevention and treatment of a variety of human disorders caused by oxidative stress. Grape seed extract enhanced the antioxidant status and decreased the incidence of free radical-induced lipid peroxidation in the central nervous system of aged rats [22, 23]. Polyphenols from grape seeds could prevent oxidative damage to cellular DNA *in vitro* [24, 25]. Guo *et al.* [26] studied the possible protective effects of grape seed oligomer and polymer procyanidin fractions against ethanol-induced toxicity. The results indicated that ethanol could induce region-specific oxidative DNA damage in which the cerebellum and hippocampus were more vulnerable, but intake of grape seed procyanidins or other natural antioxidants could protect the brain against ethanol-induced genotoxicity. Liu [27] studied the protective effects of grape seed proanthocyanidins (GSPs) against oxidative damage in brain tissue of mice. The results showed that GSP has protective effects against oxidative damage in brain tissue of mice induced by D-galactose. Olchowik *et al.* [28] indicated that the extract from grape seeds is very effective protectors

against oxidative damage in erythrocytes. Szeto *et al.* [29] studied the genoprotective effect of GSE on human lymphocytic DNA using standard and lysed cell comet assays. The results revealed that GSE significantly diminished hydrogen-peroxide-induced DNA damage in a dose-dependent manner.

The present study aims to investigate the protective potential of grape seed extracts against oxidative damage of DNA, brain and erythrocytes *in vitro*.

MATERIALS AND METHODS

Materials

Plant Material: Grape (*Vitis vinifera* L., variety Red Roumy), as large clusters with red berries, was purchased from a local market at Giza, Egypt.

Animals: Male Wistar white rats, weighing about 70-73 g were purchased from Research Institute of Ophthalmology, Giza, Egypt.

Chemicals: Ascorbic acid and ferrous sulphate were purchased from Sigma Chemical Co., USA. Calf thymus DNA was obtained from BDH Chemicals Ltd., England. Hydrogen peroxide was purchased from Loba Chemie Pvt. Ltd., India. All other chemicals were of analytical reagent grade.

Methods

Preparation of Grape Seed Extracts (GSEs): Two different extracts were prepared from red grape seeds using the procedure described by Badavi *et al.* [30] with some modifications as follows: Grape seeds were separated from the grapes manually, air dried (in shade, 25-30°C) for one week and milled to fine powder. To prepare water grape seed extract (WGSE), 0.2 g of grape seed powder was macerated in 20 ml of distilled water (DW) for 24 h at 5°C and was stirred three times. The mixture filtered with cheese cloth and the resulting filtrate was used as WGSE. The same method was used for the extraction of grape seed with ethanol 80%. After extraction with ethanol, the solvent was removed from the obtained extract by evaporation. The residue was redissolved in the same volume of DW. The obtained solution was used as ethanol grape seed extract (EGSE).

Assay of Protective Effects

Assay Against DNA Sugar Damage: The DNA sugar damage was assayed using the method described by Sultana *et al.* [31] as follows: In a clean test tubes, 0.5 ml of DNA solution (1 mg/ml of 0.15 M NaCl) was mixed with

a known volumes (0.1, 0.3 or 0.5 ml) of each grape seed extract and the mixture was completed to a known volume (1.0 ml) with phosphate buffer solution (0.1 M, pH 7.4) then 0.2 ml of ascorbic acid solution (1 mM) and 0.04 ml of ferric chloride solution (100 μ M) were added. The reaction mixture was mixed well and incubated in a shaking water bath for 1 h at 37°C. After incubation, 1.0 ml of thiobarbituric acid solution (1% in 50 mM NaOH solution) was added to the reaction mixture then kept in boiling water bath for 15 min. The Absorbance (A) was measured at 535 nm using Jenway 6300 spectrophotometer. Control was prepared by the same procedure without grape seed extract. The inhibition percent of DNA-sugar damage was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Assay Against Fe²⁺-induced Lipid Peroxidation in Brain: Fe²⁺-induced lipid peroxidation in brain was carried out using the method described by Oboh *et al.* [13] as follows:

Preparation of Brain Homogenate: Wister white rat was decapitated under mild diethyl ether anaesthesia and the cerebral tissue (whole brain) was rapidly dissected, weighed and then placed in ice. The tissue was subsequently homogenized in cold saline solution (1% NaCl, w/v) by ultrasonic probe (frequency at 10 kHz) for 30 s. The homogenate was centrifuged at 7,000 rpm for 10 min to yield a pellet that was discarded and a supernatant (S1) containing mainly water, proteins and lipids (cholesterol, galactolipid, individual phospholipids, gangliosides), DNA and RNA was kept and collected for lipid peroxidation assay.

Procedure: 100 μ l of S1 fraction were mixed with a reaction mixture containing 30 μ l of Tris-HCl buffer solution (0.1 M, pH 7.4), different volumes of grape seed extract (40, 80 or 100 μ l) and 30 μ l of freshly prepared FeSO₄ solution (250 μ M) and the volume was made up to 300 μ l by DW before incubation at 37°C for 1 h. The colour reaction was developed by adding 300 μ l of sodium dodecyl sulphate solution (8.1%, w/v). This was subsequently followed by the addition of 600 μ l of acetic acid/HCl mixture (0.1 M, pH 3.4) and 600 μ l of thiobarbituric acid solution (0.8%, w/v). This mixture was incubated at 100°C for 1 h. Thiobarbituric acid reactive species produced were measured at 532 nm. Control was prepared by the same procedure without sample. The inhibition percent of Fe²⁺-induced lipid peroxidation was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Protective Effect of Erythrocyte from Oxidative Damage:

Protection of erythrocytes from oxidative damage (*in vitro*) was carried out according to the method described by Yang *et al.* [32] with some modification as follows:

Preparation of Stock Blood Suspension: Stock blood suspension was prepared by adding a volume of whole blood to an equal volume of Alsever's solution (pH 6, glucose 2.05 g, sodium citrate 0.8 g and NaCl 0.42 g in 100 ml of DW) containing 1/30 volume of the anticoagulant solution (sodium citrate, 8.0 g, 37% formaldehyde, 54.0 ml and saline solution, 100 ml. This suspension could be stored as long as 2 weeks at 4°C.

Preparation of Erythrocytes Suspension: Erythrocytes were collected from the stock blood suspension by centrifugation at room temperature (2,000 rpm, 5 min) and washed 3-4 times with saline solution (0.9% NaCl, w/v, about 5.0 ml of saline solution for each ml of packed erythrocytes). The washed erythrocytes were added to phosphate buffer saline solution (PBS; 0.01 M phosphate buffer, pH 7.4 and 0.9% NaCl, w/v) to give a suspension 5% (about 5.0 ml of cells per 100 ml of PBS). In a clean test tubes, a serials of 3-fold dilutions of each grape seed extract were made in final volume of 1.0 ml with PBS. To each tube, 2.0 ml of erythrocytes suspension were added then incubated at 37°C for 30 min. 1.0 ml of H₂O₂ solution (10 mM) was added to each tube then incubated at 37°C for 1 h with shaken gently. Negative control was prepared by mixing 2.0 ml of PBS with 2.0 ml of erythrocytes suspension while positive control was prepared by mixing 1.0 ml of PBS, 2.0 ml of erythrocytes suspension and 1.0 ml of H₂O₂ solution. Each reaction mixture was used to determine the hemolysis percentage and lipid peroxidation.

Assay of Hemolysis: The reaction mixture (1.0 ml) was removed and centrifuged at 5,000 rpm for 2 min. The absorbance (A) of supernatant was measured at 540 nm using Jenway 6300 spectrophotometer. Reference value was determined using the same volume of erythrocytes in a hypotonic buffer (5 mM phosphate buffer, pH 7.4, 100% hemolysis). The hemolysis percentage was calculated using the following equation:

$$\text{Hemolysis (\%)} = \frac{A_{\text{sample}}}{A_{\text{reference}}} \times 100$$

Assay of Lipid Peroxidation: Lipid peroxidation was indirectly assessed through the measurement of the thiobarbituric acid reaction. One-hundred microlitres of phosphoric acid solution (0.44 M) and 250 μ l of thiobarbituric acid solution (0.67%, w/v) were added to 1.0 ml of reaction mixture and incubated at 95°C for 1 h. After cooling in an ice bath for 10 min, 150 μ l of trichloroacetic acid solution (20%, w/v) were added then centrifuged at 13,000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm using Jenway 6300 spectrophotometer. The malondialdehyde content (μ mol/ml) was calculated using molar extinction coefficient (156,000 M/cm).

Statistical Analysis: The results were analysed by an analysis of variance ($P < 0.05$) and the means separated by Duncan's multiple range test. The results were processed by CoStat computer program (1986).

RESULTS AND DISCUSSION

Several studies have shown the possible benefits of antioxidants from plant sources in altering, reversing or forestalling the negative effects of oxidative stress. The antioxidants like polyphenols, carotenoids and vitamins have an important role in the defense of cells against oxidative insult [1, 7]. The grape seed extracts under investigation were subjected to examine their protective effects against oxidative damage.

Protection Against DNA Sugar Damage: Oxidative DNA damage has been implicated to be involved in various degenerative diseases including Alzheimer's disease, Parkinson's disease, Hodgkin's disease and Bloom's syndrome [33]. The presence of various concentrations (464.4, 1339.3 and 2232.1 ppm) of each grape seed extract in the reaction mixture prevented the free radical-mediated DNA-sugar damage in a dose dependently (Table 1). The results demonstrated that there was positive relationship between the concentration of grape seed extract and inhibition of oxidative DNA damage. The maximum inhibitory effect (70.72%) was observed with EGSE at higher concentration (2232.1 ppm). However, the inhibition of DNA sugar damage reached 39.25% with WGSE at the same concentration. Noteworthy, it has been found that EGSE was also more effective than WGSE. The IC_{50} value of ethanol grape seed extract (1214.8 ppm) was lower than that of water grape seed extract (2699.5 ppm). The concentration dependent decrease in the Fenton's reaction-mediated degradation of DNA by the presence of grape seed

extracts suggest that these extracts have compounds which may combat against free radical-mediated degradation to the deoxyribose sugar moiety of DNA. The mechanism by which these extracts prevent oxidative injury to DNA, however, needs to be evaluated. It may be suggested that the antioxidant property of compounds present in extracts or metal sequestering property due to the presence of polyphenolic compounds might be responsible for such effect.

Protection Against Fe^{2+} -Induced Lipid Peroxidation in Brain: Lipid peroxides are mixture of extremely reactive products of lipid peroxidation, which is a common process in all biological systems and has deleterious effects on the cell membrane and DNA [34]. Malondialdehyde, one of the major product of lipid peroxidation has been extensively used as an index for lipid peroxidation and as a marker for oxidative stress. The reaction of MDA with TBA has been widely adopted as a sensitive assay method for lipid peroxidation [35]. In this experiment, three concentrations of each grape seed extract were used (222.2, 444.4 and 555.5 ppm). The results of the inhibitory effect of grape seed extracts on Fe^{2+} -induced lipid peroxidation in rat brain are shown in Table 2. The results clearly revealed that grape seed extracts caused a significant decrease ($p < 0.05$) in Fe^{2+} -induced lipid peroxidation in rat brain tissues in a dose dependent manner. On the same line with previous results, ethanol grape seed extract showed a higher inhibitory effect on lipid peroxidation in rat brain than water grape seed extract, at all concentrations examined. The IC_{50} value of ethanol grape seed extract was 344.5 ppm and that of water grape seed extract was 431.2 ppm. The polyphenols present in the grape seed extracts could be responsible for the inhibition of the Fe^{2+} -induced lipid peroxidation.

Protection of Erythrocytes from Oxidative Damage: In this study, normal rat erythrocytes were used to investigate the capability of the water and ethanol grape seed extracts (500-2500 ppm) to protect erythrocytes against oxidative damage *in vitro*.

Effect on H_2O_2 -Induced Hemolysis: The data of the effect of grape seed extracts (WGSE and EGSE) at different concentrations (500, 1500 and 2500 ppm) on hemolysis of rat erythrocyte induced by H_2O_2 are shown in Table 3. When erythrocytes were incubated at 37°C as a 5% suspension in phosphate buffered saline, they were stable with little hemolysis observed (15.03%). When H_2O_2 (85 ppm) was added to the suspension of erythrocytes, hemolysis induction was greatly increased (59.28%).

Table 1: Effect of water grape seed extract (WGSE) and ethanol grape seed extract (EGSE) on free radical mediated DNA-sugar damage.

Treatment	Concentration (ppm)	Inhibition%	IC ₅₀
WGSE	446.4	7.16 ^a ±0.56	2699.5
	1339.3	28.04 ^d ±0.54	
	2232.1	39.25 ^c ±1.17	
EGSE	446.4	29.75 ^d ±0.87	1214.8
	1339.3	58.57 ^b ±0.56	
	2232.1	70.72 ^a ±0.41	
LSD 0.05	--	2.258	--

Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at $P < 0.05$.

Table 2: Inhibitory effect of water grape seed extract (WGSE) and ethanol grape seed extract (EGSE) on Fe²⁺-induced lipid peroxidation in rat brain

Treatment	Concentration (ppm)	Inhibition%	IC ₅₀
WGSE	222.2	18.03 ^c ±1.89	431.2
	444.4	56.83 ^c ±1.97	
	555.5	64.48 ^b ±2.38	
EGSE	222.2	32.79 ^d ±0.95	344.5
	444.4	67.76 ^b ±1.45	
	555.5	74.86 ^a ±1.97	
LSD 0.05	--	5.627	--

Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at $P < 0.05$.

Table 3: Effect of water grape seed extract (WGSE) and ethanol grape seed extract (EGSE) on H₂O₂-induced hemolysis of rat erythrocytes

Treatment	Concentration (ppm)	Hemolysis%
Negative control (PBS)	-	15.03 ^b ±0.21
Positive control (H ₂ O ₂)	85	59.28 ^a ±0.18
WGSE	500	38.67 ^b ±0.13
	1500	28.00 ^a ±0.26
	2500	24.03 ^a ±0.13
EGSE	500	26.97 ^a ±0.17
	1500	23.15 ^a ±0.13
	2500	16.45 ^a ±0.17
LSD 0.05	--	0.534

Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at $P < 0.05$.

Table 4: Effect of water grape seed extract (WGSE) and ethanol grape seed extract (EGSE) on H₂O₂-induced lipid peroxidation of rat erythrocytes as indicated by malondialdehyde (MDA) levels

Treatment	Concentration (ppm)	MDA (μmol)
Negative control (PBS)	-	0.66 ^a ±0.009
Positive control (H ₂ O ₂)	85	2.60 ^a ±0.011
WGSE	500	1.69 ^b ±0.011
	1500	1.32 ^a ±0.006
	2500	1.13 ^a ±0.003
EGSE	500	1.29 ^a ±0.009
	1500	1.10 ^a ±0.009
	2500	0.98 ^a ±0.013
LSD 0.05	-	0.030

Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at $P < 0.05$.

Both grape seed extracts at different concentrations suppressed H₂O₂-induced hemolysis in a concentration-dependent manner. The results showed that ethanol grape seed extract significantly reduced the hemolysis percentage more than water grape seed extract at different concentrations. The hemolysis is lagged, indicating that endogenous antioxidants in the erythrocytes could trap radicals to protect them against free-radical-induced hemolysis.

Effect on H₂O₂-Induced Lipid Peroxidation: Membrane phospholipids constitute a major target for the cytotoxic effect of ROS in that their polyunsaturated fatty acids are particularly prone to peroxidation, leading to the formation of hydroperoxides, which are subsequently degraded to MDA. In order to verify the effect of grape seed extracts on the specific molecular alterations that ultimately result in oxidative hemolysis, the level of lipid peroxidation was measured. As shown in Table 4, when RBCs were treated with 85 ppm H₂O₂, a dramatic increase in MDA concentration is observable, indicating severe peroxidative damage of the RBC membrane. Pre-treatment of cells with grape seed extracts at different concentrations (500, 1500 and 2500 ppm), however, prevented the phospholipid oxidative alteration in a concentration-dependent manner. The levels of MDA were 1.29, 1.10 and 0.98 μmol with ethanol grape seed extract whilst were 1.69, 1.32 and 1.13 μmol with water grape seed extract at concentrations 500, 1500 and 2500 ppm, respectively. In controls, the level of MDA reached 0.66 μmol in negative control (with PBS) and 2.60 μmol in positive control (treated with H₂O₂ alone).

In general, the extracts of red grape seeds showed protective effects of DNA, brain and erythrocyte against oxidative damage. The protective effects of grape seed extracts could, at least partly, be due to their antioxidant and free radical scavenging properties or enhanced antioxidant capacity or their contents of polyphenolic compounds such as proanthocyanidins which have ability to reduce oxidative damage on DNA, brain and erythrocyte [21, 22, 36-40]. The higher protective effect of ethanol grape seed extract in comparison with water grape seed extract was associated with its antioxidant activity and its content of polyphenolic compounds [19, 21]. These possibilities were supported by Sangeetha *et al.* [41] who found that proanthocyanidins rich grape seed extract is an effective anti-aging drug in preventing the oxidative stress associated loss of membrane surface charge, which thereby maintains the erythrocyte

membrane integrity and functions in elderly. Chaimad *et al.* [42] found that grape seed extract showed its efficacy for brain protection from lipid peroxidation induced by high-fat diets in rats. Wang *et al.* [43] indicated that compound tianpupian (TPP) and all of its four components including extract of *Rhodiola sachalinensis*, grape seed extract proanthocyanidins, *Acanthopanax senticosus* extract and tea polyphenols had significant inhibitory activities for the oxidative damage of mouse erythrocytes, out of which the grape seed extract proanthocyanidins showed the maximal protective effect.

Finally, it could be concluded that red grape seed extracts possess a protective effects of DNA, brain and erythrocytes against oxidative damage.

REFERENCES

- Briviba, K. and H. Sies, 1994. Non Enzymatic Antioxidant Defense Systems. In Natural Antioxidants in Human Health and Diseases, Ed., B. Frie, Academic Press Inc., San Diego, CA, pp: 119-121.
- Chevion, M., 1988. A site-specific mechanism for free radical induced biological damage: the essential role of redox-active transition metals. Free Radical Biological Medicine, 5: 27-37.
- Ames, B.N., M.K. Shigenaga, T.M. Hagen, 1993. Oxidants, antioxidants and the degenerative diseases of aging. Proceedings of National Academy of Science, 90: 7915-7922.
- Anderson, D. and B.J. Phillips, 1999. Comparative *in vitro* and *in vivo* effects of antioxidants. Food Chemical Toxicology, 37: 1015-1025.
- Pardo-Andreu, G.L., S.J. Philip, A. Riano, C. Sanchez, C. Viada, A.J. Nunez-Selles and R. Delgado, 2006. *Mangifera indica* L. (Vimang) protection against serum oxidative stress in elderly humans. Archives of Medical Research, 37: 158-164.
- Arouma, O.I., 2003. Methodological consideration for characterizing potential antioxidant actions of bioactive compounds in plant foods. Mutation Research, 9: 523-524.
- Ajila, C.M. and U.J.S. Prasada Rao, 2008. Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by *Mangifera indica* L. peel extract. Food and Chemical Toxicology, 46(1): 303-309.
- Ames, B., 1989. Endogenous oxidative DNA damage, aging and cancer. Free Radical Research Communications, 7(3-6): 121-128.
- Emerit, Y. and P. Cerutti, 1981. Clastogenic activity from Bloom syndrome fibroblast cultures. Proceeding of the National Academy of Sciences, USA, 78: 1868-1872.
- Jenner, P., 1991. Oxidative stress as a cause of Parkinson's disease. Acta Neurologica Scandinavica, 84: 6-15.
- Desmarchelier, C., E. Mongelli, J. Coussio and G. Ciccial, 1997. Inhibition of lipid peroxidation and iron (II)-dependent DNA damage by extracts of *Pothomorphe peltata* (L.) Miq. Brazilian Journal of Medical and Biological Research, 30: 85-91.
- Shulman, R.G., D.L. Rothman, K.L. Behar and F. Hyder, 2004. Energetic basis of brain activity: Implications for neuroimaging. Trends in Neuroscience, 27(8): 489-495.
- Oboh, G.R.L. Puntel and J.B.T. Rocha, 2007. Hot pepper (*Capsicum annum*, Tepin and *Capsicum chinese*, Habanero) prevents Fe²⁺-induced lipid peroxidation in brain *in vitro*. Food Chemistry, 102(1): 178-185.
- Lam, R.Y.Y., A.Y.H. Woo, P.S. Leung and C.H.K. Cheng, 2007. Antioxidant actions of phenolic compounds found in dietary plants on low-density lipoprotein and erythrocytes *in vitro*. Journal of the American College of Nutrition, 26(3): 233-242.
- Widen, C., A. Ekholm, M.D. Coleman, S. Renvert and K. Rumpunen, 2012. Erythrocyte antioxidant protection of rose hips (*Rosa* spp.). Oxidative Medicine and Cellular Longevity, Article ID 621579, pp: 8.
- Yoo, M.A., H.K. Chung and M.H. Kang, 2004. Evaluation of physicochemical properties in different cultivar grape seed waste. Food Science and Biotechnology, 13: 26-29.
- Bagchi, D., M. Bagchi, S.J. Stohs, D.K. Das, S.D. Ray, C.A. Kuszynski, S.S. Joshi and H.G. Pruess, 2000. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology, 148: 187-197.
- Rapport, L. and B. Lockwood, 2001. Proanthocyanidins and grape seed extract. The Pharmaceutical Journal, 266: 581-584.

19. Hassan, H.M.M. and N.M.M. Hassan, 2010. *In vitro* antioxidant and free radical scavenging activities of red grape seed extracts. Global Journal of Biotechnology and Biochemistry, 5(2): 106-115.
20. Tang, Q., P. Zou, H. Jin, J. Fu, J. Yang, L. Shang and X. Wei, 2012. Grape-seed proanthocyanidins ameliorate contact hypersensitivity induced by 2, 4-dinitrofluorobenzene (DNFB) and inhibit T cell proliferation *in vitro*. Toxicology Letters, 210: 1- 8.
21. Hassan, H.M.M., 2012. Hepatoprotective effect of red grape seed extracts against ethanol-induced cytotoxicity Global Journal of Biotechnology and Biochemistry, 7(2): 30-37.
22. Balu, M., P. Sangeetha, D. Haripriya and C. Panneerselvami, 2005. Rejuvenation of antioxidant system in central nervous system of aged rats by grape seed extract. Neuroscience Letters, 383: 295-300.
23. Devi, A., A.B. Jolitha, N. Ishii, 2006. Grape seed proanthocyanidin extract (GSPE) and antioxidant defense in the brain of adult rats. Medical Science Monitor, 12(4): 124-129.
24. Fan, P.H. and H.X. Lou, 2004. Isolation and structure identification of grape seed polyphenols and its effects on oxidative damage to cellular DNA. Yao Xue Xue Bao, 39(11): 869-75.
25. Balu, M., P. Sangeetha, G. Muralli and C. Panneerselvami, 2006. Modulatory role of grape seed extract on age-related oxidative DNA damage in central nervous system of rats. Brain Research Bulletin, 68: 469-473.
26. Guo, L., L.H. Wang, B. Sun, J.Y. Yang, Y.Q. Zhao, Y.X. Dong, M.I. Spranger and C.F. Wu, 2007. Direct *in vivo* evidence of protective effects of grape seed procyanidin fractions and other antioxidants against ethanol-induced oxidative DNA damage in mouse brain cells. Journal of Agricultural and Food Chemistry, 55(14): 5881-5891.
27. Liu, Y.M., 2009. Protective effects of grape seed proanthocyanidins against oxidative damage in brain tissue of mice. Food Science, 30(21): 374-376.
28. Olchowik, E., K. Lotkowski, S. Mavlyanov, N. Abdullajanova, M. Ionov, M. Bryszewska and M. Zamaraeva, 2012. Stabilization of erythrocytes against oxidative and hypotonic stress by tannins isolated from sumac leaves (*Rhus typhina* L.) and grape seeds (*Vitis vinifera* L.). Cellular and Molecular Biology Letters, 17(3): 333-348.
29. Szeto, Y.T., K.Y. Lee, W. Kalle and S.C. Pakd, 2013. Protective effect of grape seed extracts on human lymphocytes: a preliminary study. Applied Physiology, Nutrition and Metabolism, 38(3): 275-279.
30. Badavi, M., F.Z. Mchrgerdi and A. Sarkaki, 2008. Effect of grape seed extract on lead induced hypertension and heart rate in rat. Pakistan Journal of Biological Sciences, 11(6): 882-887.
31. Sultana, S., S. Perwaiz, M. Iqbal and M. Athar, 1995. Crude extracts of hepatoprotective plants, *Solanum nigrum* and *Cichorium intybus* inhibit free radical mediated DNA damage. Journal of Ethnopharmacology, 45: 189-192.
32. Yang, H.L., S.C. Chen, N.W. Chang, J.M. Chang, M.L. Lee, P.C. Tsai, H.H. Fu, W.W. Kao, H.C. Chiang, H.H. Wang and Y.C. Hseu, 2006. Protection from oxidative damage using *Bidens pilosa* extracts in normal human erythrocytes. Food and Chemical Toxicology, 44: 1513-1521.
33. Imlay, J.A. and S. Linn, 1988. DNA damage and oxygen radical toxicity. Science, 240(4857): 1302-1309.
34. Kubow, S., 1995. Routes of formation of toxic consequences of lipid oxidation products in foods. Free Radical Biological Medicine, 12: 63-81.
35. Ohkawa, H., N. Ohishi and K. Yagi, 1978. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95: 351-358.
36. Bagchi, D., A. Garg, R.L. Krohn, M. Bagchi, D.J. Bagchi, J. Balmoori and S.J. Stohs, 1998. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation and peritoneal macrophage activation in mice. General Pharmacology, 30(5): 771-776.
37. Carini, M., G. Aldini, E. Bombardelli, P. Morazzoni and R.M. Facino, 2000. UVB-induced hemolysis of rat erythrocytes: Protective effect of procyanidins from grape seeds. Life Sciences, 67: 1799-1814.
38. Kim, H.J. Deshane, S. Barnes and S. Meleth, 2006. Proteomics analysis of the actions of grape seed extract in rat brain: Technological and biological implications for the study of the actions of psychoactive compounds. Life Sci., 78: 2060-2065.
39. De Rezende, A.A.A., U. Graf, Z.R. Guterres, W.E. Kerr and M.A. Spano, 2009. Protective effects of proanthocyanidins of grape (*Vitis vinifera* L.) seeds on DNA damage induced by Doxorubicin in somatic cells of *Drosophila melanogaster*. Food and Chemical Toxicology, 47: 1466-1472.

40. Praphasawat, R., P. Klungsupya, T. Muangman, S. Laovitthayangoon, V. Arunpairojana and L. Himakoun, 2011. Antimutagenicity and antioxidative DNA damage properties of oligomeric proanthocyanidins from Thai grape seeds in TK6 cells. *Asian Pacific Journal of Cancer Prevention*, 12: 1317-1321.
41. Sangeetha, P., M. Balu, D. Haripriya and C. Panneerselvam, 2005. Age associated changes in erythrocyte membrane surface charge: Modulatory role of grape seed proanthocyanidins. *Experimental Gerontology*, 40: 820-828.
42. Chaimad, U., S. Pongshompoo and S. Srichairat, 2009. Effect of grape seed extract on lipid peroxidation and brain damage induced by high-fat diet in rats. *Thai Journal of Pharmacology*, 31(1): 87-90.
43. Wang, Y.X., R.B. Wei, Z. Feng and S.Y. Cui, 2011. Protective effects of compound tianpupian against oxidative damage in mouse erythrocytes. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*, 19(1): 215-218.