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Grape extract protect against ionizing radiation-induced DNA damage

Indrani Singha[1], Sudhanshu Saxena[2], Satyendra Gautam[2], Abhijit Saha[3] & Subir Kumar Das[1]*

[1]Department of Biochemistry, College of Medicine & JNM Hospital, The West Bengal University of Health Sciences, Kalyani, Nadia-741 235, West Bengal, India

[2]Food Technology Division, Bhabha Atomic Research Centre, Mumbai- 400 085, Maharashtra, India

[3]UGC-DAE-CSR, Bidhan Nagar, Kolkata-700 098, West Bengal, India

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Grape extracts of different cultivars (Flame seedless, Kishmish chorni, Red globe and Thompson seedless) were investigated for *in vitro* antioxidant activity by ABTS assay, and compared protective efficacy against radiation-induced DNA damage. Seed extract showed the highest scavenging activity, followed by skin extract. Among different cultivars, 'flame seedless' skin extract showed higher scavenging activity followed by 'Kishmish chorni' skin extract. Grape extracts significantly prevented radiation-induced plasmid DNA damage. Super-coiled pBR 322 plasmid DNA (~93%) is completely converted to open circular (~97%) and linear (~2%) form at a dose of 150 Gy γ-radiation. Pretreatment with different grape extracts showed various degree of protection against radiation-induced DNA damage. Pretreatment with 1.6 µg grape skin extract of 'Thompson seedless' cultivar or grape flesh extract of any tested cultivar diminished the DNA strand breaks, and there was an increase in the super coiled form of DNA against 150 Gy of γ-radiation. However, pretreated pBR 322 DNA with the skin of 'Kishmish chorni' cultivars or seed of 'red globe' grape cultivars remained static during electrophoresis and confined in the groove on exposure to 150 Gy γ-radiation treatment. Co-treatment with the skin of red globe cultivar also partially confined plasmid DNA in the groove. The same trend was observed when plasmid DNA was exposed to 1.2 kGy γ-radiation. Our investigation revealed that anthocyanin present in grape skin was probably involved in radio protective activities through the formation of co-pigmentation with DNA.

Keywords: Anthocyanin, Flavilium, Hemiketal, Ionizing radiation, Oxonium ion

Ionizing radiation (IR) is used for diagnostic as well as in therapeutic medical applications and some industrial purposes[1]. IR is an effective and commonly employed treatment in the management of more than half of human malignancies[2]. In some cases, radiation may be the single best treatment for cancer[3]. However, the detrimental effects of IR are not restricted only in the irradiated cells, but also to a non-irradiated bystander or even distant cells manifesting various biological effects[1].

The energy absorbed by a biological system due to exposure to radiation triggers a number of successive events including damage to living tissues[4]. As approximately 90% water is present in cells, IR exposure leads to oxidizing events in the cell through direct interactions of radiation with target macromolecules or *via* products of water radiolysis[5]. During the radiolysis of water, hydroxyl radicals ($OH^{\bullet}$), hydrogen atom (H), hydrated electron (e_{aq}-), hydrogen peroxide (H_2O_2), *etc*. are produced. Under hypoxic conditions as seen in tumors, the formation of other species such as superoxide (O_2^-) and singlet oxygen[6] are also significant. These free radicals act as molecular marauders and in turn damage DNA, which is considered to be the most critical target regarding the biological effects of IR on cells[4]. The DNA of the IR-exposed cells undergoes single- strand breaks, double-strand breaks, and damage to the bases and sugars, DNA-protein cross-links, and ultimately leading to chromosomal aberrations[7]. In addition, the generation of ROS brings about oxidation of critical cellular machinery leading to lipid peroxidation in cellular membranes and protein oxidation[8-10], resulting in cellular dysfunction and death[11].

Antioxidant enzymes which form the major first-line defense in response to radiation are an important factor in the manifestation of radiation effects[11]. Many

*Correspondence:
Phone: +91-9432674673 (Mob)
E-mail: drsubirkdas@gmail.com

Abbreviations: ABTS, 2, 2-azino-bis (3-ethylbenzenethiazolin-6-sulphonic acid) diammonium sulphate; FRAP, Ferric reducing antioxidant power assay; IR, Ionizing radiation; MA, Monomeric anthocyanin; TAE, Tris-acetate-EDTA

natural and synthetic chemicals have been tested to determine their protective efficacy against radiation-induced damages in biological systems[12-18]. However, due to the inherent toxicity of some of the synthetic agents, the practical applicability of the majority of these synthetic compounds remained limited at the radioprotective concentration[7]. Therefore, the evaluation of natural compounds as radioprotective agents has attracted much attention due to their non-toxic nature and easy availability. Plant products with various pharmacological properties; have been used for the treatment of various diseases since time immemorial. Therefore, screening of various plants and their active principals offers a major focus for the discovery of new radioprotective drugs[19].

Grapes, one of the most popular, widely cultivated and consumed fruits in the world[20], are rich in phytochemicals[21]. These are responsible for nutraceuticals and health benefits[22]. It is well known that the grape skins, seeds, and stems, are rich sources of polyphenols, including flavonoids, phenolic acids, and stilbenes[23]. *In vitro* and *in vivo* studies have established that grape consumption is related to the prevention of chronic diseases such as cardiovascular diseases[24], cancer[25, 26], diabetes[27], obesity[28], platelet aggregation[29], and hypertension[30]. However, phytochemical composition and their effects vary significantly among the different grape cultivars[20, 31-34]. Recently we have reported on the scavenging properties and cellular protective actions of grape extracts of different cultivars against oxidative stress generated by H_2O_2 and Fenton-like reagent in *ex vivo* system[31, 32].

However, reports on DNA protective activity of grape extracts are quite limited. As radiation effects are mainly mediated by oxidative mechanisms and grape extracts have ameliorative effects against oxidative stress, hence we evaluated whether the grape extracts are able to protect against radiation-induced plasmid pBR322 DNA damage, and the possible mechanism of radioprotection.

Materials and methods

Materials

Plasmid DNA pBR322 was purchased from M/s Bangalore Genei, India. All other analytical reagents were purchased from Merck India Ltd. or SRL India,

Grapes

Commonly available four grape (*Vitis vinifera* L.) cultivars, including 'Flame seedless' (black), 'Kishmish chorni' (black with reddish-brown), 'Red globe' (red) and 'Thompson seedless' (Sonaka, Green) were purchased from the local market and authenticated from the Department of Fruits and Orchard Management, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal. Damaged or diseased fruits were eliminated. The fruits were lyophilized after removing the skin, flesh, and seed, packaged and were stored at −20°C.

50 mg of each sample was mixed in 500 µL miliQ water, centrifuged and the supernatant was separated for analysis.

Methods

ABTS [2, 2-azino-bis (3-ethylbenzenethiazolin-6-sulphonic acid) diammonium sulphate] assay

The antioxidant in the sample converts the $ABTS^+$ radical to ABTS (colorless). ABTS (7 mM; 10 mg in 2.6 mL milliQ water) was mixed with ammonium persulfate (2.45 mM; 1.45 mg in 2.6 mL ABTS) and the solution was kept in dark at ambient temperature (~26°C) for more than 16 h. This produces $ABTS^+$ in solution. The concentration of the ABTS solution was optimized at an absorbance value of ~0.7 at 734 nm. Different concentrations of aqueous grape extracts were added in 1 mL of ABTS solution, and absorbance was recorded after 6 min using spectrophotometer at 734 nm. 0.1 mL of milliQ water in 1 mL of ABTS solution was used as blank. IC_{50} of each sample was calculated[35].

$$\text{Inhibition}(\%)=\frac{(\text{Absorbance of Control}-\text{Absorbance of Sample})}{\text{Absorbance of Control}}\times 100$$

pBR322 plasmid DNA relaxation assay for DNA damage

In vitro DNA protective capacity of grape extract using pBR322 plasmid DNA was evaluated following the method described elsewhere[36] with modification. Two microlitre of pBR322 plasmid DNA (180 µg/ mL) 1XPBS (pH 7.4) (Bangalore Genei, India) was added to 16 µL of grape extracts in a microfuge tube (Eppendorf, 1.5 mL), and irradiated at a dose 150 Gy (γ cell-220) and 1.2 kGy (γ cell-5000), respectively, at a dose rate 3.4 Gy/ min at Food Technology Division, Bhabha Atomic Research Centre, Mumbai, India. Irradiated DNA solution without grape extracts served as the experimental control. Non-irradiated DNA solution without grape extracts served as the control. All samples were subjected to agarose gel (1.2%) electrophoresis. Agarose gel electrophoresis was also performed using TAE (Tris–acetate–EDTA) buffer (pH 8.0) (100 mM Tris-acetate, 2 mM EDTA buffer (pH 8.3).

The gels were stained with ethidium bromide (0.5 µg/mL) and visualized using a UV-trans-illuminator. The gel image was taken by the gel doc system (Alpha Innotech Corp) and the bands were quantified.

Agarose gel electrophoresis was also performed using TAE (Tris–acetate–EDTA) buffer (pH 8.0) (100 mM Tris-acetate, 2 mM EDTA buffer (pH 8.3).

DNase treatment

50 µL each of skin fractions from 'Thompson seedless' and 'Kishmish chorni' cultivars were subjected to boiling water bath (20 min in a water bath) followed by cooling at room temperature.

16 µL of each extract was taken in microfuge tubes (1.5 mL), 2 µL of pBR322 DNA was added in each sample, incubated for 40 min at room temperature.

Samples treated by 2X TAE Buffer

16 µL each of skin fractions from 'Thompson seedless' and 'Kishmish chorni' cultivars were mixed separately with 1 µL 50X TAE was added to each sample to make the final conc. of 2X TAE, 2 µL of DNA was added to each sample and were incubated for 40 min at room temperature. Samples treated with Tris-Cl (*p*H 8)

16 µL each of skin fractions from 'Thompson seedless' and 'Kishmish chorni' cultivars were mixed with 0.25 µL Tris-Cl (12.5 mM, pH-8). 2 µL of DNA was added to each sample and were incubated for 40 min at room temperature.

Samples treated with EDTA

16 µL each of skin fractions from 'Thompson seedless' and 'Kishmish chorni' cultivars were mixed with 0.4 µL EDTA (1 mM). 2 µL of DNA was added to each sample. The samples were incubated for 40 min at room temperature.

Clean-up through PCR clean up kit

A GenElute minprep binding column was inserted (with a blue o-ring) into a collection tube (SIGMA). 0.5 mL of the column preparation solution was added to each miniprep column, centrifuged at 15000 rpm for 10 min (20-25°C) and the elute was discarded. 32 µL of skin extract from 'Thompson seedless' or 'Kishmish chorni' cultivar was mixed with 4 µL of DNA and was incubated for 40 min at room temperature. This 36 µL of sample solution was mixed with 200 µL binding solution and transferred to the binding column. The column was centrifuged at 15000 rpm for 10 min, and the eluent was discarded, while the collection tube was retained. The binding column was replaced into the collection tube, 0.5 mL of diluted wash solution was mixed to the column, centrifuged at maximum speed for 1 min, the elute was discarded and the collection tube was retained. The column was replaced into the collection tube and centrifuged the column at maximum speed for 2 min, without any additional wash solution, to remove excess ethanol. Any residual elutes as well as the collection tube was discarded. The column was transferred to a fresh 2 mL collection tube. 15 µL of elution solution (pH was maintained between 5.5 and 8.5) was applied to the center of each column, incubated at room temperature for 1 min. The pure DNA solution was collected by centrifuging the column at 15000 rpm for 1 min. Before loading on the gel 3.5 µL of DNA loading was mixed with each sample and electrophoresis was performed. Images were analysed using GelQuant.NET software.

Extraction and purification of anthocyanin from 'Kishmish chorni' (black) grape skin:

'Kishmish chorni' grape skin mixed with acidified methanol (containing 0.3% HCl) followed by thorough vortexing. Then it was passed through a 0.45 µM filter (Millex). One mL sample was collected in each microfuge tube (1.5 mL) and kept it in vacuum drier (Eppendrof concentrator 5301) until it dried. 1 mL of acidified miliQ was added to this dried sample, mixed well, centrifuged for 10 min in 10000 rpm and the supernatant was collected.

The column was washed with 5 mL of methanol, and then with 10 mL of acidified miliQ (0.01% HCl) water. Then anthocyanin- rich extract was loaded into the column. The column was washed with 20 mL acidified miliQ water followed by 5 mL of ethyl acetate. Then the anthocyanin- rich extract was eluted with 4 to 6 mL of acidified methanol (0.01% HCl). 5 mL elute was collected in two microfuge tubes with anthocyanin- rich extract and evaporated to dryness.

TLC Analysis

1 mL of acidified methanol was added to the extract of purified anthocyanin samples and were subjected to thin- layer chromatography using the solvent containing butanol: acetic acid: water (4:1:5). After the run, the TLC plates were kept in dark until the plates dry and later analysed.

Total monomeric anthocyanin (TMA) estimation

Monomeric anthocyanin (MA) content was determined by the AOAC pH differential method. Briefly, the absorbance of diluted grape extracts at

aqueous buffer at pH 1.0 buffer (potassium chloride, 0.025 M: 1.86 g KCl in ~ 980 mL distilled water, and adjust pH to 1.0 (±0.05) with HCl (~ 6.3 mL), and dilute final volume to 1 L with distilled water.) and sodium acetate buffer at pH 4.5 buffer (sodium acetate, 0.4 M: 54.43 g CH_3CO_2Na, $3H_2O$ in ~960 mL distilled water, adjust pH to 4.5 (±0.05) with ~ 20 mL HCl, and final volume was made to a 1 L with distilled water) were determined at 510 and 700 nm after 15 min equilibration against distilled water blank. The MA was calculated as follows:

$$MA = \frac{[(A_{510\,nm} - A_{700\,nm})\ pH\,1.0 - (A_{510\,nm} - A_{700\,nm})\ pH\,4.5] \times MW \times DF \times (10^3\ mg/g)}{\varepsilon \times l}$$

where A is Absorbance, MW is molecular weight and DF is dilution factor, ε is molar extinction coefficient, and l is path length (in cm).

In order to measure MA as cyaniding-3-glycoside equivalent (CGE), the amount of MA was calculated using the molar absorptivity (ε = 26,900 in $Lmol^{-1}cm^{-1}$), and molecular weight (MW = 449.2), and 10^3 = factor for conversion from g to mg. The amount of MA was then expressed as mg CGE/100 g fresh weight (fw)[37].

Statistical analysis

Results are reported as mean ± standard deviation (SD) of three independent experiments. Data analysis was performed using GraphPad Prism software 5, and *P* value <0.05 was considered as significant.

Results

The experimental data depicts the concentration-dependent decolourization of $ABTS^{o+}$, expressed as IC_{50} values, by grape extracts in comparison with standard antioxidant ascorbic acid (Table 1). Seed extract showed highest scavenging activity. Among edible part, skin extract showed stronger scavenging activity compared to pulp extract, and 'Flame seedlees' skin extract showed higher scavenging activity followed by 'Kishmish chorni' skin extract scavenging activity (Table 1).

Exposure of plasmid DNA to γ radiation resulted in production of strand breaks. We have evaluated radioprotective efficacy of different colored cultivars and different parts (seed, skin and pulp) of grapes; *i.e.* extracts from different parts of 'Kishmish chorni' (Black with reddish brown colour), 'Thompson seedless' (green colour) and 'Red globe' cultivars. Super coiled pBR 322 plasmid DNA (~93%) is completely converted to open circular (~97%) and linear (~2%) form at an optimised dose of 150 Gy γ-radiation (Fig. 1, Lane 2). Differential protection to plasmid DNA was observed by different grape extracts. Pretreatment with 1.6 µg grape skin extract of 'Thompson seedless' cultivar (Fig. 1, Lane 3) or grape flesh extract of any tested cultivar (Fig. 1, Lanes 4, 6 and 8) reduced the formation of DNA strand breaks as a result of which there was an increase in supercoiled form of DNA against 150 Gy of γ-radiation. However, pretreated pBR 322 plasmid DNA with skin of 'Kishmish chorni' cultivar or seed of 'Red globe' grape cultivars did not move through the wells and confined in the groove after 150 Gy radiation treatment (Fig. 1, Lanes 5 and 10). Partial holding up of pBR 322 plasmid DNA after 150 Gy radiation was observed while it was pretreated with the skin of red globe cultivars (Fig. 1, Lane 7).

Table 1 —*In vitro* effects of grape extracts from different cultivars in terms of inhibitory concentration (IC_{50}= µg/ mL) on ABTS activity

Grape cultivars		ABTS radical scavenging
Thompson seedless (Green)	Skin	500.6 ± 30.7*
	Pulp	1106.3 ± 43.2
Flame seedless (Black)	Skin	82.3 ± 12.4*
	Pulp	653.3 ± 29.6
Kishmish chorni (Black with Reddish Brown)	Skin	91.1 ± 19*
	Pulp	714.2 ± 29.1
Red globe	Skin	112 ± 16.4*
	Pulp	912.3 ± 25.3
	Seed	58 ± 15*
Ascorbic acid		31.6 ± 2

Values are mean ± standard errors (SE) of means of three experiments

P values: *<0.05 compared to pulp within same cultivar

While DNA was subjected to normal electrophoresis with or without skin extracts of different grape cultivars, DNA pretreated with the skin of 'Thompson seedless' cultivar moved through the gel in its native form (Fig. 2, Lane 2), while others did not move (Fig. 2, Lanes 3 and 4). Heating of skin extract of 'Kishmish chorni' cultivar did not facilitate in the mobility of the DNA (Fig. 3, Lane 7), however, TAE (Tris-acetate-EDTA) treatment to the skin of 'Kishmish chorni' cultivar induced movement of the DNA through electrophoresis (Fig. 3, Lane 8). Further treatment of 'Kishmish chorni' with Tris-Cl (12.5 mM) assisted in the movement of DNA (Fig. 4, Lane 2), but not by EDTA (1 mM) (Fig. 4, Lane 3). 'Kishmish chorni' skin treated DNA was confined in the groove and did not move during electrophoresis; but treatment with Tris-Cl and TAE, the movement of DNA was observed during electrophoresis.

Further exposure of DNA to 1.2 kGy γ-radiation completely degraded the DNA, while different grape extracts offered protection by retaining them in super-coiled form or holding them in the groove as observed during 150 Gy γ-radiation (Fig. 5).

While pretreated DNA with skin of 'Thompson seedless' cultivar or 'Kishmish chorni' cultivar passed through PCR clean up kit and subjected to electrophoresis, 'Thompson seedless' skin treated DNA showed electrophoreses (Fig. 6, Lane 1) but not 'Kishmish chorni' skin treated DNA (Fig. 6, Lane 2).

Qualitative TLC analysis showed the presence of anthocyanin in the skin extract of 'Kishmish chorni' grape cultivar (Fig. 7).

Total monomeric anthocyanins ranged from 270 mg/kg of fresh weight of grape skin (fw) for 'Kishmish chorni', followed by in 'Flame Seedless' (228 mg/ kg fresh weight) to 76 mg/kg of fw for 'Thompson Seedless'; while small amount of anthocyanins were estimated in pulp (Table 2).

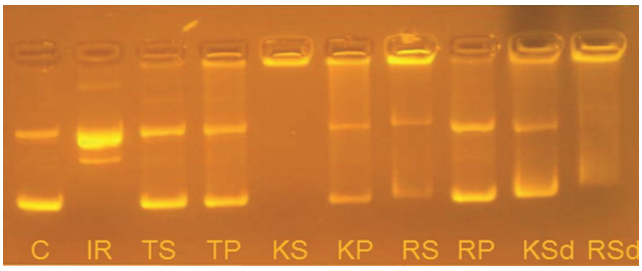

Fig 1 — Electrophoretogram of pBR 322 DNA after 150 Gy γ radiation in the absence or presence of the pretreated grape extract of different cultivars. T: Thompson seedless, K: Kishmis chorni, R: Red globe; S: Skin; P: Pulp; Sd: Seed

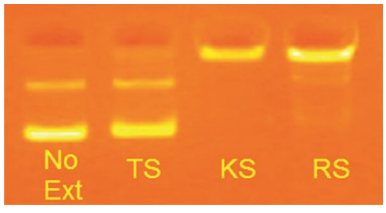

Fig. 2 — Effects of grape skin extract on pBR322 after incubation for 45 min. No extract was used in the control set DNA pretreated with the skin of green cultivars moved through the gel in its native form (Lane 2), while others did not move (Lane 3) or marginally moved (Lane 4)

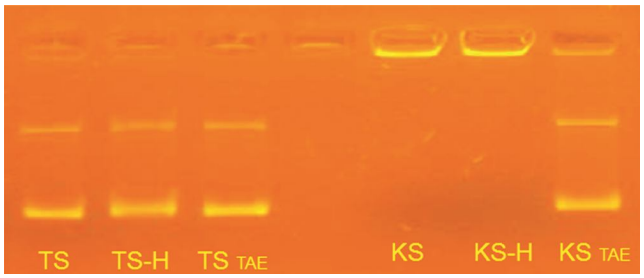

Fig. 3 — Effect of Heat and TAE Treatment of grape skin on pBR 322 DNA. H: Heated in boiling water bath for 25 min; TAE: Conc. 2X. Heating of skin extract of black cultivar did not help in mobility of the DNA (Lane 7), however, TAE (Tris acetate-EDTA) treatment to the skin of black cultivar helped in the movement of the DNA through electrophoresis (Lane 8)

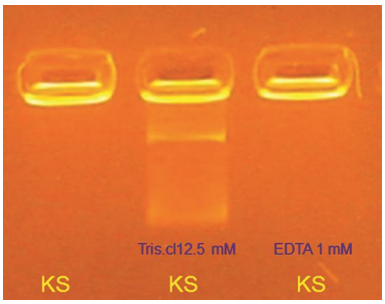

Fig. 4 — Effect of different treatment on grape skin at pH 8.0 on pBR322 DNA. Further treatment of black skin with Tris-Cl (12.5 mM) helped in the movement of DNA (Lane 2), but not by EDTA (1 mM) (Lane 3)

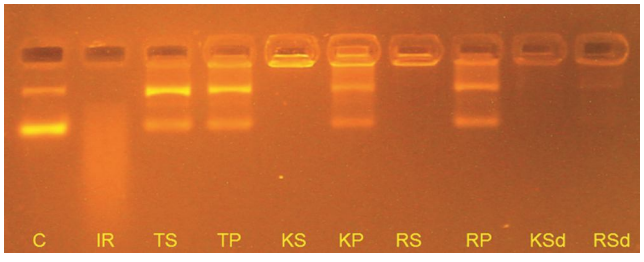

Fig. 5 — Effect of grape extract pretreatment to pBR 322 DNA, prior to 1.2 kGy γ radiation. Complete DNA degradation occurs at 1.2 kGy. C: Non-radiation, IR: 1.2 kGy γ radiation, T: Thompson seedless, K: Kishmis chorni, R: Red globe; S: Skin; P: Pulp; Sd: Seed

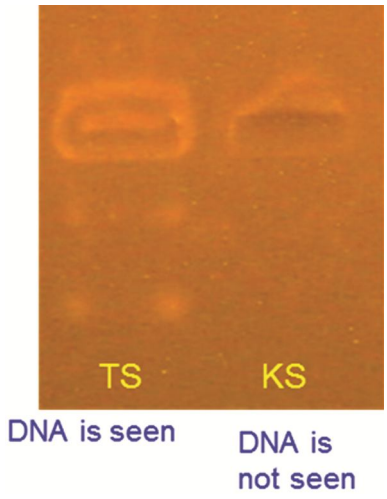

Fig. 6 — Skin of green cultivar and black cultivar were pretreated to the DNA, subjected to pass through PCR clean up kit and subjected to electrophoresis, green skin treated DNA was electrophoreses (Lane 1) but not black skin treated DNA (Lane 2)

Table 2 — Total monomeric anthocyanin (TMA) content of grape extracts from different cultivars

Grape cultivars		TMA content (mg cyanidin-3-glucoside equivalents equivalent/100 g fresh weight)
Thompson seedless (Green)	Skin	76 ± 6.7
	Pulp	18 ± 2.3
Flame seedless (Black)	Skin	228 ± 14.6
	Pulp	22 ± 9.6
Kishmish chorni (Black with Reddish Brown)	Skin	270 ± 12.3
	Pulp	16 ± 4.3
Red globe	Skin	124 ± 6.4
	Pulp	17 ± 2.3
	Seed	ND

Values are mean ± standard errors (SE) of means of three experiments; ND: Not done

Discussion

Grape berries contain three major tissue types: skin, pulp, and seed. The skin contains volatile and nonvolatile and color compounds, the pulp contribute organic acids and sugars, and the seed provides condensed tannins[38]. Distribution of polyphenols in the berry is inconsistent, and ~60–70% of total soluble phenolics are found in the seed, ~28–35% in the skin, and only ~10% in the pulp[39].

ABTS is a measure of antioxidant activity, in contrast to antioxidant concentration that might include a proportion of biologically-inactive antioxidants[40,41]. The highest ABTS radical scavenging potential was shown by seed extract, followed by skin and pulp. Studies have shown that polyphenols and their derivatives more effectively scavenge the ABTS radicals[42, 43]. This is in agreement with our reported studies[31-34].

As DNA is the major target of radiation, the ability of grape extracts to prevent radiation-induced DNA damage was investigated using plasmid DNA (pBR322). Considering our previous results[31-34], we have evaluated the efficacy of grape extracts of 'Kishmish chorni', 'Thompson seedless', 'Red globe' and 'Flame seedless' cultivars. The induction of single-strand break in plasmid DNA results in conversion of the super-coiled form of DNA into an electrophoretically slow migrating, open- chain form, and further to linear forms[44]. The electrophoretic pattern revealed that the grape extracts prevented DNA from damage by γ irradiation in our study (Fig. 1 & Fig. 5). Pretreatment with 1.6 µg grape extracts reduced the DNA strand breaks as evidenced by an increase in the supercoiled form, with subsequent decrease in open circular form of DNA, indicating recovery of super coiled form.

Our results further showed that components present in black skin grape cultivar bound to the DNA and did not allow the movement of DNA during electrophoresis. Heating of this extract for 25 min, did not show electrophoresis separation of DNA, indicating that DNase had no possible role in retaining this DNA. Interestingly, treatment with TAE resulted in DNA movement. While treatment with Tris-Cl (pH 8) allowed movement of the DNA, but EDTA treatment did not. These observations demonstrated that possible chelating effect on DNase has no role, but the pH of the medium is effectively responsible for movement during electrophoresis. It is now known that anthocyanins are responsible for a wide range of colors in many plant organs, including grape berries[45] and are characterized by a positive charge on the molecule[46]. Possibly positively charged anthocyanin strongly bound with negatively charged DNA and converts it into a heavier form that protected it from movement, resulting in confinement into the groove. But, treatment with pH-dependent Tris- Cl and TAE, the binding of grape extracts with DNA was weakened, which resulted in dissociation of the DNA, and allowed its movement through the gel.

It has been reported that anthocyanins protect DNA against oxidative damage by forming a co-pigmentation complex[47]. In this study, co-pigmentation properties of anthocyanin together with scavenging properties might

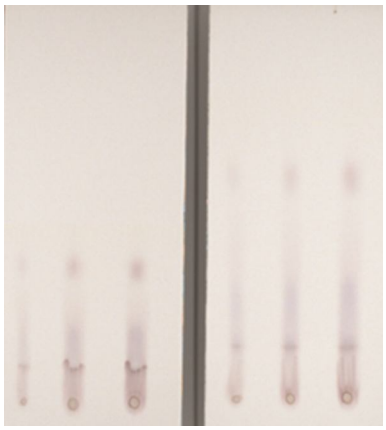

Fig. 7 — Qualitative TLC analysis (under white light)of anthocyanin enriched fraction

have shielded the DNA from the oxidative damage by γ radiation (Fig. 8). Studies have also argued that the stability of intermolecular copigmentation complexes was highly sensitive to pH[48].

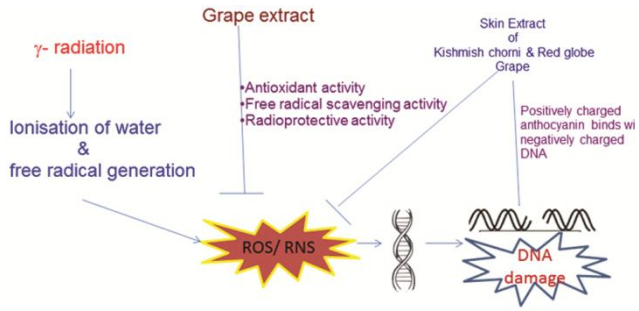

Fig. 8 — Proposed mechanism of action of grape extract in response to gamma radiation-induced damage

The pH dependence of anthocyanin is further utilized in its estimation in our study. Anthocyanins forms colored flavylium (oxonium) cation at a pH of 1.0 having a maximum absorbance at a wavelength of 510 nm[49,50]. However, a proton is lost from the anthocyanin at pH 4.5, resulting in a colorless carbinol pseudobase (hemiketal form) structure[50,51]. The accumulation of water-soluble flavonoid pigments anthocyanins in grape skins is greatly influenced by cultivars[52] as observed in this study.

Though most studies showed the accumulation of anthocyanins only in the skin of many grape cultivars, few studies reported the flesh in low quantity[53-56].

Conclusion

While different grape extracts protected DNA from by γ radiation-induced damage at different extent in this study; however, skin extracts of the 'Kishmish chorni' and 'Red globe' cultivars demonstrated potent antioxidant activities, for their possible free radical-scavenging effect. Grapes having black or red color skin that protected DNA from γ-radiation contain higher concentration of anthocyanins. This water-soluble flavonoid pigments anthocyanins in grape skin protected DNA from radiation-induced damage through co-pigment formation.

Acknowledgement

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Conflict of Interest

All authors declare no conflict of interest.

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