CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com Research Article

# THE STUDY OF ANTIOXIDANT ACTIVITY OF BLACK GRAPES EXTRACT TO PREVENT DNA DAMAGE

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**Abstract:**

*Introduction: The side products of cellule metabolism like ORSs can damage DNA alkalis and cause restrain in simulation. To avoid any kind of damages and disturbances in cellules they should be controlled and eliminated. Active kinds which are produced in stress oxidation can cause damages to DNA and so they are mutagenic which can prevent cellule dying, increase in reproduction, invasion and metastasis. Different fruits' juices especially pomegranate juice is studied as anti-oxide synthesis that cause better anti-oxide condition and prevent DNA break. Yet black grape juice is a natural one that contains the most beneficent group of flavonoids and pro anti cyanide oligomers. Grape (vitis vinifera) is one of the most different fruits that are used fresh or processed. The stone and peel of black grape contains active compounds specially flavonoid, anthocyanidin, pro anthocyanidin, pro cyanidin and resveratrol that are strong eliminators for free radical. Because of the importance of genome protection, in this study the protective effect of black grape juice as a resource of anti-oxidant in controlling the DNA break is discussed. Materials and Methods: pBR322 plasmid and a nucleotide sequence which is reproduced from ITGB3 gene has been chosen as a model of PCR output. Some kinds of DNA were exposed to different density of black grape juice. Results: According to results of this study, the best concenteration of black grapes juice for prevention of DNA damage of genomic DNA and plasmid pBR322 were 3.100 and 1.100 respectively. Conclusion: The results indicatedthat the antioxidant activity of black grapes juice can inhibit the production of free radicals and DNA damage.*

**Key words:** *DNA damage, Fenton reaction, black grape juice, ITGB3 gene, Antioxidant activity, Free radical*

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*Please cite this article in press as Sareh Rahimi et al.,* ***The study of antioxidant activity of black grapes extract to Prevent DNA damage****, Indo Am. J. P. Sci, 2017; 4(11).*

## INTRODUCTION:

Free radicals in the cells are produced by cellular metabolism and by exogenous agents including ionizing radiation and carcinogenic compounds. One of the sources of free radicals production is Fenton's reaction in the body. These radicals react with biomolecules in cells and one of their main goals is DNA. This damage is also called oxidative damage to DNA, which has consequences in many organs, and especially in the brain, due to metabolic activity and high oxygen consumption. These consequences include mutagenesis, carcinogenesis and prevention. Mutagenicity occurs at varying degrees, from simple oxidation of the cells to large deletions through single-stranded and double-stranded DNA fractures (7, 4). Hydroxyl radical, a hydrogen atom of the deoxyribose ribose picks, and with it, a strand of DNA can cause failure (10). This brain damage if not repaired neurodegenerative brain diseases such as Alzheimer's or Parkinson's leads. A number of mutagenic, leading to structural changes in cellular DNA, which accidentally led to changes in the properties of DNA and cytotoxicity are produced code (7). Fruits are the natural source of antioxidants, including anthocyanins and polyphenolic compounds, and they can reduce the risk of developing neurodevelopmental diseases such as cancer, diabetes and cardiovascular disease, which are the result of oxidative stress. Red fruits such as sour cherries, red grapes, barberry and pomegranate Follows:

The two primers replicate a 424 nucleotide fragment from the ITGB3 glycoprotein IIIA glycoprotein IIIA gene.

are rich in these types of compounds (7). Grape juice is a rich antioxidant source. In this study, the direct effect of grape juice on inhibiting oxidative damage to DNA was investigated by the reaction of Fenton induced by iron at specified times and different concentrations of grape juice, and tested using electrophoresis gel method Took.

**Grape juice**

Grape juice was commercially available from San Echo Company. In this study, Fenton digestive activity was performed on extracted DNA from healthy blood samples and plasmid pBR322. Using two ITGB3 primers, genomic DNA was propagated by PCR reaction. The antioxidant properties of grape juice were then evaluated to control the failure of these DNAs. DNA fracture was then investigated by PCR and gel electrophoresis.

## RESULTS:

Design Primer

To reproduce the desired component, a suitable primer pair is required that adheres to both sides of that piece. Primers used in this study included a primer pair for exon 3, which was designed using the GenRuner application. To perform a PCR reaction from two forward primers (F) and reverse (R) were used.

The sequence of these two primers is as

F: 5` GGATTATCCCAGGAAAGACCA 3`
R: 5` GACTTCCTCCTCAGACCTCCA 3`

**Table 1: Characteristics of primers used**.

| Primer | Primer length (pairwise) | Sequences | Product length PCR (bp) |
|---|---|---|---|
| forward | 21 | 5'-GGATTATCCCAGGAAAGACCAC-3' | 424 |
| Reverse | 21 | 5'-GACTTCCTCCTCAGACCTCCAC-3' | |

To use primers in the PCR technique, you must first dilute them. Thus, the main stack of primers F and R was each removed at a rate of 2.5 µl and poured into a microtib one 1.5 ml And washed with 100 ml of sterile water for later use The freezer was stored at -20 ° C (if primers F and R were designed to complement each other and form a diimer-primer, they should be diluted in two separate microtitudes).

**Table 2: content of materials required for Fenton digestive reaction in PCR product**

| Sample number / Materials required | 1(Control) | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| product PCR | 5µL | 5µL | 5µL | 5µL | 5µL | 5µL |
| $H_2O_2$(50mM) | - | 2µL | 2µL | 2µL | 2µL | 2µL |
| $Fe(SO_4)_{20}$ $7H_2O$ (3mM) | - | 2.5µL | 2.5µL | 2.5µL | 2.5µL | 2.5µL |
| EDTA(10mM) | - | 1.5µL | 1.5µL | 1.5µL | 1.5µL | 1.5µL |
| Phosphate buffer (PH= 7.4 0.1 M) | 1.5µL | 1.5µL | 1.5µL | 1.5µL | 1.5µL | 1.5µL |
| $H_2O$ | 13.5µL | 7.5µL | 6.5µL | 5.5µL | 4.5µL | 3.5µL |
| grape juice | - | - | 1µL | 2µL | 3µL | 4µL |
| Final content | 20µL | 20µL | 20µL | 20µL | 20µL | 20µL |

**Table 3: Final concentration of the required materials for the Fenton digestive reaction in the product of PCR**

| Sample number / Materials required | 1 (Control) | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $H_2O_2$ | - | 5mM | 5mM | 5mM | 5mM | 5mM |
| $Fe(SO_4)_{20}$ $7H_2O$ | - | 0.375mM | 0.375mM | 0.375mM | 0.375mM | 0.375mM |
| EDTA | - | 0.75 mM | 0.75 mM | 0.75 mM | 0.75 mM | 0.75 mM |
| Phosphate buffer | 7.5mM | 7.5mM | 7.5mM | 7.5mM | 7.5mM | 7.5mM |

Antioxidant effect of grape juice on DNA fragmentation inhibition by extracting PCR after digestion reaction:

First, the PCR product was diluted 20 times. Grape juice was also diluted 5 times and control samples and other samples At 6 micrometers, 1.5 units were prepared separately and placed inside the incubator at 37 ℃ for two hours. After digestion, each of the specimens was diluted 40 times. Then, for these samples, the RCR20 program was repeated for the PCR reaction cycle. Then the agarose gel was loaded at 1.5% concentration and electrophoresed at 100 volts.

**Table 4: content of materials required for Fenton digestive reactions in PCR**

| Sample number / Materials required | 1 )Control( | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| product PCR 20 times diluted | 2µL | 2µL | 2µL | 2µL | 2µL | 2µL |
| $H_2O_2$(50mM) | - | 2µL | 2µL | 2µL | 2µL | 2µL |
| $Fe(SO_4)_{20}$ $7H_2O$ (3mM) | - | 2.5µL | 2.5µL | 2.5µL | 2.5µL | 2.5µL |
| EDTA(10mM) | - | 1.5µL | 1.5µL | 1.5µL | 1.5µL | 1.5µL |
| Phosphate buffer (PH= 7.4 0.1 M) | 1.5µL | 1.5µL | 1.5µL | 1.5µL | 1.5µL | 1.5µL |
| $H_2O$ | 16.5µL | 10.5µL | 9.5µL | 8.5µL | 7.5µL | 5.5µL |
| grape juice | - | - | 1µL | 2µL | 3µL | 4µL |
| Final content | 20µL | 20µL | 20µL | 20µL | 20µL | 20µL |

**Table 5: Final concentration of materials for Fenton digestive reaction in PCR**

| Sample number / Materials required | 1 (Control) | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $H_2O_2$ | - | 5mM | 5mM | 5mM | 5mM | 5mM |
| $Fe(SO_4)_{20}\,7H_2O$ | - | 0.375mM | 0.375mM | 0.375mM | 0.375mM | 0.375mM |
| EDTA | - | 0.75 mM | 0.75 mM | 0.75 mM | 0.75 mM | 0.75 mM |
| Phosphate buffer | 7.5 mM | 7.5mM | 7.5mM | 7.5mM | 7.5mM | 7.5mM |

The effect of fenton reaction on pBR322 plasmid and the antioxidant effect of grape juice on its inhibitory effect in 30 minutes:

Circular DNA was commercially available from Sinoclon Co. (Tehran). Pouring the necessary ingredients into the microtubules was carried out quickly and on ice to prevent possible damage to the plasmid before the material is placed in an incubator at 37 ℃.

First, inside the microtubes Phosphate buffer, EDTA. Then a solution of iron (II) sulfate (oxygen) and finally a plasmid was added and distilled with water twice The content was 20µL. microtubes were slowly spin until the material was completely mixed and a uniform solution was obtained. microtubes were then incubated at 37 ℃ for 30 minutes.

**Table 6: the volume of materials needed for the Fenton digestive reaction on the plasmid pBR322**

| Sample number / Materials required | 1 (Control) | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Plasmid pBR322 | 1µL | 1µL | 1µL | 1µL | 1µL | 1µL |
| $H_2O_2$(50mM) | - | 2µL | 2µL | 2µL | 2µL | 2µL |
| $Fe(SO_4)_{20}\,7H_2O$ (3mM) | - | 2.5µL | 2.5µL | 2.5µL | 2.5µL | 2.5µL |
| EDTA(10mM) | - | 1.5µL | 1.5µL | 1.5µL | 1.5µL | 1.5µL |
| Phosphate buffer (PH= 7.4 0.1 M) | 1.5µL | 1.5µL | 1.5µL | 1.5µL | 1.5µL | 1.5µL |
| $H_2O$ | 17.5µL | 11.5µL | 10.5µL | 9.5µL | 8.5µL | 7.5µL |
| grape juice | - | - | 1µL | 2µL | 3µL | 4µL |
| Final content | 20µL | 20µL | 20µL | 20µL | 20µL | 20µL |

**Table 7: Final concentration of the required material for the fenton digestive reaction on the plasmid pBR322**

| Sample number / Materials required | 1 (Control) | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $H_2O_2$(50mM) | - | 5mM | 5mM | 5mM | 5mM | 5mM |
| $Fe(SO_4)_{20}$ 7H $_2$O (3mM) | - | 0.375mM | 0.375mM | 0.375mM | 0.375mM | 0.375mM |
| EDTA(10mM) | - | 0.75 mM | 0.75 mM | 0.75 mM | 0.75 mM | 0.75 mM |
| Phosphate buffer (PH= 7.4 0.1 M) | 7.5 mM | 7.5mM | 7.5mM | 7.5mM | 7.5mM | 7.5mM |

The concentration of gel electrophoresis is determined by the size of the parts to be separated the pBR322 plasmid has a size of 4361 bp. Therefore, for electrophoresis of the plasmid and its fragments, the agar gel was used at 0.8%. This concentration of gel can separate pieces from 1000 to 7000 bp. For 0.8% agarose gel, 0.16 g of agarose powder was weighed and 20 ml TBE buffer was added. Here it took an hour to close the gel at the laboratory temperature until the gel was completely tightened. After 30 minutes, samples were removed from the incubator. The 10 µL of each sample was mixed with µL4 from buffer lining and loaded into the wells. At this stage, it is so large sized that it stops at the bottom of the DNA ladder, so there was no need for a DNA ladder. Because the study is qualitative and the conclusion is based on eye observations, full precision should be taken to remove the specified volume of material and to load the material into the wells to prevent handicap. When loading the specimens, the Microtubes were placed on ice and the specimens were carefully loaded onto the gel. The gel was placed inside the electrophoresis tank and the tank was filled with a TBE buffer to cover the surface of the gel. The specimens were then loaded into the wells. Since the DNA sample is at this stage of the plasmid test, the sample time in the gel was considered for 3 hours. Using the 100 volt for 3 hours causes the gel to warm up and create a smiley state in the bands. Therefore, the electrophoresis tank was connected to a 50-volt Powerslence for 3 hours. After electrophoresis, the gel was transferred from the electrophoresis tank to the glass plate and with diluted etymid bromide Colored for 20 minutes. Note etymium bromide it is a mutagenic substance and it must be taken care of. The gel was then placed inside the dye gel and gel was taken by adjusting the UV light intensity.

Electrophoresis result of PCR of the ITGB3 gene

DNA was extracted from four healthy human blood samples. The ITGB3 was amplified by PCR and finally electrophoresed. The result of electrophoresis is shown in the figure below, The result of electrophoresis is shown in the figure below, the measurement marker was used to determine the size of the amplified DNA bands. Sharp bands, no extra band and smear were used for further experiments.

Figure 1 Electrophoresis of the PCR gene of ITGB3

Lines 1 through 4, PCR product of the ITGB3 gene in 4 human and L-DNA samples: DNA Lader (100 bp)

## DISCUSSION AND CONCLUSION:

Free radicals in the cells are produced by cellular metabolism and by exogenous agents including ionizing radiation and carcinogenic compounds. These radicals react with biomolecules in cells, and one of their main goals is DNA, which has implications for many organs, especially in the brain, due to metabolic activity and high oxygen consumption. These include mutagenicity, carcinogenesis and aging. Mutagenicity occurs at varying degrees, from simple oxidation of the cells to large deletions through double-stranded DNA fractures (7, 4). A radical hydroxylic acid removes a hydrogen atom in the deoxyribose, and thus it breaks down a string of DNA (10). The reaction of fenton is the reaction of iron ions with hydrogen peroxide, in other words, the oxidation of iron substrate by iron (II) hydrogen peroxide is called fenton reaction.

Certain metallic ions with a particular oxidation number, especially Fe2 + and Cu +, by catalyzing the reaction of fenton, lead to the production of highly active and dangerous hydroxyl radicals. The reaction of Fenton induced by Fe2 + metal ions is a classic Fenton reaction, in which Fe3 + is produced (8).

$$Fe\ (II) + H_2O_2 \longrightarrow Fe\ (III) + HO^- + HO^{\cdot}$$

Many studies in animal and human models have shown that phenols play a protective role against oxidative stress and free radical damage. Antioxidants alone neutralize the radicals or activate other molecules for this purpose (3). The results of this study indicate that grape juice inhibits Fenton's response due to the presence of antioxidant properties in an effective dose-dependent manner. This inhibition is accomplished by neutralizing $H_2$ $O_2$ or radical OH cleansing produced during the Fenton reaction. In a study, Rezvan et al. Investigated the antioxidant properties of water and leaf extract of some sugar cane genotypes by DPPH radical purification assay It was found that in some genotypes leaf extract, and in others, cane sugar has a more radical purification effect. They investigated the protective effect of water and leaf extracts of sugarcane genotype on extracted DNA from sugarcane leaves against hydroxyl radicals produced during the Fenton digestive reaction by gel electrophoresis technique. It was concluded that both leaf extract and cane sugar can protect DNA from the reaction of the fenton (2). In another study, Schneider and colleagues conducted a study on the properties and mode of damage of the plasmid pBR322 from free radical hydroxyl. They used the ascorbate / iron system in a phosphate buffer. It was then concluded that ascorbate in the vicinity of iron leads to the production of free radical hydroxyl. Radical hydroxyl production in this system caused the plasmid DNA to break down (9). Ajit also investigated the effect of ginger antioxidant on the breakdown of pBR322 plasmid and DNA extracted from the liver and brain tissue of the mouse by Fenton reaction in the presence of Fe + 2 and $H_2$ $O_2$ by gel electrophoresis technique. The results of this study showed that ginger extract was prevented by the antioxidant property of oxidative failure of pBR322 plasmid and the DNA extracted from the liver and brain tissue of the mouse was induced by the reaction of Fenton (1). In addition, Kumar et al. Have the protective effects of extracts extracted with chloroform and ethyl acetate from a golden rainbow leaf called Scientific Colertria Paniculata Laksom Investigate the failure of pUC18 plasmid by Fenton reaction with gel electrophoresis technique. Both types of extraction of golden rainbow leaves prevented the damage to DNA from the plasmid pUC18 and reduced its defeat by the Fenton reaction (11). In the present study, the results of the first and second experiments indicate that re-PCR is required for treated DNA samples to reveal the effect of Fenton reaction on agarose gel. Since the Fenton reaction causes a DNA string or two strands to fail in a random manner, and this failure is not visible in the DNA molecular molecule on the agarose gel, to detect the amount of DNA damaged, samples diluted in an incubator for 2 hours, diluted 1 to 40, and again PCR. In PCR again, samples whose DNA is damaged by the Fenton reaction will not multiply, and ultimately, depending on the number of DNA molecules that remain healthy, we will have PCR product. The more DNA molecules are damaged, the less we will have after the PCR. This difference was observed in the number of healthy DNA molecules by loading PCR reagents on agarose gel and comparing the intensity of the bands on the gel. Also, the results of the second experiment show that up to about 4 cc of grape juice is added, there is a direct relationship between the concentration of grape juice and the control of the Fenton reaction. As the concentration of grape juice increased gradually, inhibition of DNA damage induced by the Fenton reaction was further increased, but an inverse relationship was observed at higher concentrations. We conclude that according to recent studies, the higher the antioxidant concentration used in the reaction, the more the DNA fragmentation is inhibited, and thus the direct relationship between the concentration of grape juice and the control of the Fenton reaction is justified, but to justify the inverse relationship observed, we consider for some reason that as follows:

Reducing the reaction pH by increasing the amount of grape juice

The reaction of Fenton is performed at an acidic pH. Because in acidic conditions $H_2O_2$ is more protected. The best pH for the Fenton reaction is pH 3-5. Gradually adding more grape juice to the samples, pH of 7.4 changed to acidic pH (4, 12). In these acidic conditions, the Fenton reaction is furthermore done. Under these acid conditions, Fenton's response is most likely. Polyphenols also have prooxidant properties, and not only do not have antioxidant properties to protect DNA from the Fenton reaction, but also lead to further damage to the DNA.

**Acknowledgment**
This article is extracted from my thesis under the title of "The study of antioxidant activity of black grapes extract to Prevent DNA damage". Hereby, I extend my sincere appreciation to Islamic Azad university of Ashkezar for the efforts and supports they provided to me.

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