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EVALUATION OF ANTIOXIDANT PROPERTIES CHERRY FRUIT EXTRACT ON PREVENTION THE FAILURE OF DNA

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Abstract:

Free radicals preface can result in many diseases such as atherosclerosis, heart diseases, geriatrics and cancer. These diseases are resulting from irregular production of ROS and unstable mechanism of antioxidant protection system.in order to reduction of harmful effects of ROS, natural antioxidants of plants and synthesis antioxidant can be used. It is shown that phenyl compounds, lipid peroxidations and oxidative damage to DNA in some plants can be prevented through cleaning some ROS such as Hydrogen peroxide and hydrocele radicals.

Black Cherry is brimful of these compounds; in addition, root and stem of black cherry are used in medical profession.

Since genome protection is necessary for demonstration of disease prevention resulting from genome damage, in this study protection in of black cherry as a source of antioxidant in prevention of DNA break is considered.

In this study pbr322 plasmid and a nucleotide sequence reproduced from NOS gene were chosen as a sample of PCR product. DNA samples were placed in the vicinity of black cherry juice different density.

Conclusion of black cherry juice with density of 10% volume had the most prevention influence on plasmid break.

Black cherry juice in both vicinity of $\frac{1}{15}$ *and* $\frac{1}{25}$ *volume in time of 1 hour prevented PCR product break.*

Our results in this study show that black cherry juice prevents DNA break and destruction of it.

Upper vicinity of black cherry juice have been shown that it is possible , because of PH changes , presence of vitamin c and usefulness of peroxide phenyl compounds exist.

Key words: *DNA damage, Fenton reaction, black cherry juice, nitric oxide.*

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INTRODUCTION:

Antioxidants are substances that prevent the formation of free radicals in the cells. Antioxidants play an important role in preventing cancer. One of the most important natural sources of antioxidants is medicinal plants. Medicinal plants are rich in phenolic compounds. The most important sources of natural antioxidants in the human diet are legumes, plants and fruits. Natural antioxidants that are present in these sources protect the human body from free radicals and oxidative stress. These antioxidants play an important role in human health. Various studies have found that a diet rich in fruits, vegetables, or legumes plays an essential role in controlling chronic diseases such as heart disease and certain cancers through the freezing of free radicals. Some herbs have been reported to contain antioxidant compounds such as bioflavonoids, proanthocyanidins, phytoestrogens, which have shown protective and anti-cancer properties. It has been shown that these compounds inhibit oxidative stress and carcinogenesis through several mechanisms. [10]. in some plants, it has been shown that phenolic compounds inhibit lipid peroxidation and oxidative damage to DNA by purifying ROSs such as hydrogen peroxide and hydroxyl radicals. There are two main mechanisms for dealing with free radicals, one of which is the non-enzymatic antioxidant system, such as polyphenols, ascorbic acid and carotenoids.[4] Exposure to various chemical and structural contaminants such as herbicides, insecticides, toxic chemical waste, cigarette smoke, stress, sunlight and diet have similar effects on human health. [14] These contaminations ultimately lead to the oxidation of lipids, proteins, and DNA, which in some cases activates carcinogens and leads to cancer, and in some cases, is associated with aging of the cell and other malformations. Oxidative stress plays a role in neurodegenerative diseases such as Parkinson, Alzheimer and Huntington, as well as multiple sclerosis. Examining biomarkers like reactive oxygen species and nitrogen or RNS, as well as antioxidant systems, shows that oxidative damage is involved in the pathogenesis of these abnormalities. Oxidative stress is associated with some cardiovascular disease as well as the progression of various cancers. The genome of cells is constantly being damaged, which is inevitable due to DNA damage caused by natural cellular processes. By-products of cell metabolism such as ROS can damage the DNA region and inhibit replication. In order to avoid any disruption or damage to the cell, this damage should be prevented and resolved. Active species produced in oxidative stress cause damage to DNA and are therefore mutagenic, which can cause cell death, proliferation, invasion and metastasis. [3] Currently, many synthetic antioxidants have toxic and carcinogenic effects on human health. Natural antioxidants, such as L-ascorbic acid, are widely used for immunity, but have less antioxidant activity than synthetic antioxidants. A lot of attention has been drawn in recent decades to finding natural ingredients in foods or herbs to replace synthetic antioxidants. On the other hand, polyphenols, which are used as natural antioxidants, have become very important because of their health benefits for humans to reduce the risk of cardiovascular disease and disease-prone illness. [7]. Antioxidants can purify or inhibit active oxygen species by using their alienating properties, preventing damage by binding to metal ions and inhibiting hydroxyl production, as well as lipid hyperoxidation and DNA damage. There are many antioxidants in nature that are different in terms of physical and chemical structure. They include enzymes such as catalase and peroxidases, high molecular weight compounds such as albumin, low molecular weight compounds such as tocopherols, and the latter include plant compounds. [6]

Cherry Extract

Many herbal compounds and extracts have been used as antioxidants. In addition, there are many edible fruits that are used as natural and oral antioxidants. For example, grapevine extract, pomegranate, lemon and sour cherry are among the most important natural antioxidants, with numerous studies on their properties. [6] Cherry is one of the fruits that is predominantly fresh in markets around the world. Cherry is a good source of phenolic compounds that are considered to be health. Ascorbic acid, flavonoids, and especially anthocyanins are the main components of the cherry extract, which have a great potential for protecting human health. Anthocyanins in cherries are found mainly in the skin. Cranberry products have recently attracted much attention because of its beneficial effects on health such as antioxidant activity, nerve cell defense and cancer-control properties. These effects are due to its antioxidant compounds, which play an important role in reducing the oxidative damage and free radicals. Several studies have linked cancer inhibition to its antioxidant effects. [5, 9] Free radicals can lead to many heart diseases such as atherosclerosis, aging and cancer. These diseases result from irregular ROS production and an unbalanced mechanism of antioxidant system protection. To reduce the harmful effects of ROS, you can use natural antioxidants from plants and synthetic antioxidants. However, the use of these compounds has some dangers.

MATERIALS AND METHODS:

PCR was used in this research. For PCR, it is necessary to mix DNA patterns, primers, and materials needed to perform a PCR reaction in a microtiful. The materials used for the PCR reaction can be used either individually or as a Master Mix kit. In this study, Master Mix [Yekta Takiz Azma] was used. The kit contains materials such as the Taq polymerase enzyme, dNTP, PCR buffer, MgCI2 and other uncertain volumes, which facilitates the PCR reaction. The final volume of the PCR mixture should be 25 µl, so after mixing the material with the appropriate volume, the remaining volume was added to reach 25 µl of distilled water. The PCR mixture was prepared for each sample according to the following table1.

Table 1: Materials required for PCR reaction

ingredients	Volume of each sample µl	Final concentration of the material
Master Mix[2x]	12.5	1X
Primer F and R5.2]μM [	2	0.2 μM
H_2O	7.5	-
DNA Pattern	3	-
Final volume	25	-

Table 2: Thermal PCR Program for NOS 3

Temperature [° C]	Time	Repeat [cycle]
95	5 minutes	Initial denaturation
95	30 seconds	20 cycles
62.5	30 seconds	
72	30 seconds	

Table 3: Volume of materials required for Fenton digestive reactions in PCR

Sample number / Materials required	1 [Control]	2	3	4	5
PCR product is diluted 20 times	$2\mu L$	$2\mu L$	$2\mu L$	$2\mu L$	$2\mu L$
$H_2O_2(50\text{mM}]$	-	$2\mu L$	$2\mu L$	$2\mu L$	$2\mu L$
$Fe(SO_4)7H_2O_2$ $(3mM)$	-	$2.5\mu L$	$2.5\mu L$	$2.5\mu L$	$2.5\mu L$
EDTA [10mM]	-	$1.5\mu L$	$1.5\mu L$	$1.5\mu L$	$1.5\mu L$
Phosphate buffer 0.1M and PH=7.4	$1.5\mu L$	$1.5\mu L$	$1.5\mu L$	$1.5\mu L$	$1.5\mu L$
H_2O	$16.5\mu L$	$10.5\mu L$	$9.5\mu L$	$8.5\mu L$	$7.5\mu L$
Cranberry juice			1	$2\mu L$	$3\mu L$
Final volume	$20\mu L$	$20\mu L$	$20\mu L$	$20\mu L$	$20\mu L$

Table 4: Final concentration of the required material for Fenton digestive reaction in PCR

Sample number / Materials required	1 [Control]	2	3	4	5
$H_2O_2(50\text{mM}]$	-	5mM	5mM	5mM	5mM
$Fe(SO_4)7H_2O_2$ $(3mM)$	-	0.375 mM	0.375 mM	0.375 mM	0.375 mM
EDTA [10mM]	-	0.75mM	0.75mM	0.75mM	0.75mM
Phosphate buffer 0.1M and PH=7.4	$7.5\mu L_{\text{mM}}$	7.5mM	7.5mM	7.5mM	7.5mM

All the steps of mixing the ingredients for PCR should be performed on the ice to prevent the early activation of the Taq polymerase enzyme and the creation of side products after pouring primers. And template DNA. Also, all the tools and materials needed to perform PCR should be sterile so that there is no problem in the reaction due to contamination. The PCR mixtures were also closed after tight preparation the samples were then placed in a thermo cycler and, according to the program given to the thermocycler machine, PCR cycles were performed and the DNA pattern was replicated high and used for further experiments.

Agarose gel electrophoresis

Electrophoresis of the agarose gel is required to determine the correctness of the PCR and amplification of the desired part.

Perform PCR reaction

All of the PCR reactions were performed in a volume of 25 µl with a Peqlab device.

Perform PCR Reaction

In order to investigate the effect of cherry extract on preventing DNA fragmentation, digestion product was used in PCR reaction. To do this, in the 5 new microtitudes, we added the ingredients for the PCR reaction in the table below. We added 3 microlitres of 20-fold diluted digestion to each of the microtutes.

All steps for mixing the ingredients for PCR should be performed on the ice so that the early activation of the Taq polymerase enzyme and the creation of side products after pouring out the primers and pattern DNA. Also, all the tools and materials needed to perform PCR should be sterile so that there is no problem in the reaction due to contamination. The PCR mixtures were also closed after tight preparation, and then the specimens were placed in a thermo cycler and, according to the program given to the thermo cycler, The PCR cycles were performed and the DNA pattern was replicated to a large extent and used for further experiments.

Exercise the antioxidant effect of cherry juice on inhibition of DNA fragmentation by re-PCR

First, the PCR product was diluted 20 times. Cranberry juice was also 5 times diluted and according to Table 3, control samples and other samples At 5 micrometers, 1.5 separate, prepared for incubation at 37 ℃ for two hours. After digestion, each sample was diluted 40 times. Then for these samples, with the same previous PCR program, but this time With 20 cycles, PCR reaction was performed again. The agarose gel was then loaded at a concentration of 1.5% and electrophoresed at 100 volts. The final concentration of substances is given in the table 3.

RESULTS:

The result of electrophoresis of the PCR of the NOS3 gene

DNA was extracted from four healthy human blood samples, NOS3 gene was amplified by PCR and finally electrophoresed The result of electrophoresis is shown in Fig. 1 The DNA Ladder was used to determine the size of the amplified DNA bands. The resulting Sharp bands were found to be free of any excess bandages. The combination of this product was used for further experiments.

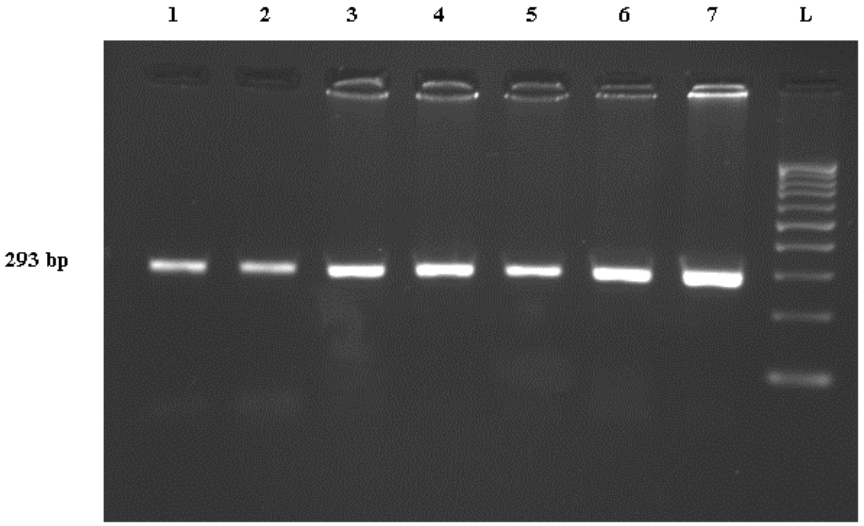

Fig 1- Electrophoresis image of the PCR product of the NOS3 gene. Lines of 1 to 7 blood samples from healthy individuals.

L line: DNA LADDER [bP 100]

DISCUSSION:

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 consequences in many organs, and in particular in the brain, in terms of metabolic activity and high oxygen consumption. These include mutagenicity, carcinogenesis and aging. The mutagenesis varies widely from simple oxidation of bases to large debris through single-stranded and double-stranded DNA fractures [7, 8]. A radical hydroxylic acid removes a hydrogen atom in the deoxyribose, and thus it breaks down a string of DNA [9]. The reaction of fenton is the reaction of iron ion with hydrogen peroxide and other oxidation of iron substrate by iron [II] hydrogen peroxide to the reaction of fenton. Certain metal ions with a particular oxidation number, especially Fe2 + and Cu +, catalyze the reaction of Fe2 +, lead to highly reactive hydroxylamine radical production. Fenton reaction induced by Fe2 + metal ion is a classic Fenton reaction, in which Fe3 + is produced [9].

$Fe\ [II] + H_2O_2 \quad Fe\ [III] + HO^- + HO^{\cdot}$

The cherry extract is a natural source of antioxidants, including anthocyanins and polyphenolic compounds, and can reduce the risk of diseases such as neurodevelopmental diseases, cancer, diabetes and cardiovascular disease, which are oxidative [11,14].] Many studies in animal models and humans 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 [10].] Many studies in animal models and humans 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 [10]. The results of this study indicate that cranberry juice inhibits Fenton's response due to its antioxidant properties in a dose-dependent manner. This inhibition is accomplished by neutralizing H_2 O_2 or radical OH cleansing produced during the Fenton reaction. [10 and 11]. During the study, Rezvan et al. investigated the antioxidant properties of the water and leaf extract of some sugar cane genotypes by DPPH radical purification measurements. It was found that in some genotypes leaf extract and in other cane juice had more radical purification property. They investigated the protective effect of water and sugarcane extract on the DNA extracted from radical hydroxyl produced during the Fenton digestive tract by gel electrophoresis technique. They concluded that both leaf extract and cane sugar can protect DNA from the reaction of the fenton [12]. The nuclear genome, and in particular the mitochondrial genome, are always exposed to oxidative stress. In spite of the antioxidant defense systems in the cell, active species sometimes escape from this immune system, resulting in oxidative damage to all cellular components, including DNA. In addition to internal factors, external factors such as air pollution and UV light, as well as pathophysiological conditions such as inflammation and genetic defects, lead to an increase in the production of free radicals in the cell. Polyphenols are compounds that have antioxidant properties. Feeding Polyphenols can protect the cell against oxidative damage. Cherry is a fruit rich in phenolic compounds. The reaction of Fenton is a reaction that infects a variety of damage to cellular components, including DNA. [12 and 13] The present study confirms previous studies that increased iron [II] and H_2O_2 concentrations increase the oxidative damage to DNA. Also, the exposure of DNA to iron [II] and H_2 O_2 for a long time caused more oxidative damage, which was observed with increasing incubation time from 35 minutes to 90 minutes for plasmid and from one hour to two hours for PCR product. . Cranberry juice was investigated in three different concentrations in a plasmid test as an inhibitor of Fenton's reaction. The present experiment showed that cranberry juice could prevent DNA damage by fenton reaction. However, increasing the concentration of cherry juice does not have an increased effect on inhibiting oxidative damage to DNA, but has the most inhibitory effect at a certain concentration. Cranberry juice was used for plasmid dilution 1 to 5 and 1 to 10 which at 5% vol / vol concentration had the most inhibitory effect at both 35 and 90 minutes In the case of cherry juice on the product of PCR, cherry juice was prepared at dilutions of 1 to 5 and 1 to 10, both of which had an inhibitory effect on DNA defects at dilution for one hour. And within 2 hours dilution of 1 to 10 with a volume / volume of 0.01 prevented DNA from being destroyed. Cranberry juice prevents the oxidation of DNA by radical hydroxyl as a rich source of antioxidants. By increasing the concentration of cherry juice, its antioxidant properties decreased in DNA protection this can be due to acidification of the pH of the reaction environment, the presence of vitamin C in cherry juice and the pro-oxidant properties of phenolic compounds in the acidic reaction environment under Invitro conditions. 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 strand 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 that were placed within the incubator for two hours, It was diluted 1 to 40 and PCR again. In PCR, samples whose DNA is damaged by the Fenton reaction will not be replicated and ultimately, depending on how many DNA molecules remain healthy, we will have PCR products. The more DNA molecules are damaged eventually, after PCR, we will have a smaller product. 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. The results of the third experiment show that up to 4 µl of cherry juice is added; there is a direct relationship between the concentration of cherry juice and the control of the Fenton reaction. Thus, with increasing cranberry juice concentrations, inhibition of DNA fracture induced by 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 greater the inhibition of DNA, and thus the direct relationship between the concentration of cherry juice and the control of the Fenton reaction is justified.

CONCLUSION:

Extract of antioxidant-rich plants, if used as a brain substance, reduces oxidative stress and, consequently, the risk of neurodegenerative diseases. Polyphenols are among the compounds that have antioxidant properties. Feeding polyphenols can protect the cell against oxidative damage. Cherry is rich in phenolic compounds. This study expresses the antioxidant and cherry juice protection of genomic DNA and the pBR322 plasmid against damage. . These properties of cherry juice, it introduces it as a source of natural antioxidants with the potential for reducing oxidative stress and ultimately maintaining health. According to the results of this study, the best concentrations of cherry juice were obtained in order to prevent genomic DNA fragmentation at $\frac{1}{15}$and $\frac{1}{25}$ hours, and at 15 to 15 hours, the best concentrations of cherry juice were obtained to prevent plasmid defeat the pBR 322 was 10% vol / vol.

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