IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 4, Issue 2 (Mar. - Apr 2018), PP 41-44
www.iosrjournals.org

Investigation Of Oxıdatıve Dna Damage Preventıon Actıvıty Of Almond (A. Trichamygdalus) Fruıt Wıth Copper And Wıthout Copper

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Abstract:
Purpose: *Almond is a fruit that can be grown and consumed by almost every region of the world. For many years people have thought that almond juice is good for many diseases. As a result of the studies carried out today, it has been determined that the certain amount of used rubbein is good for cancer, diabetes and many diseases mainly due to its antioxidant properties.This study investigated whether A. trichamygdalus fruit, an almond species, is beneficial to human health. It is known that the DNA of the main cause of the above-mentioned diseases is damage. In this study, it was aimed to investigate whether the A. trichamygdalus fruit had an effect on preventing or correcting DNA damage. It has been very important to study the effects of the substances that have recently been linked or interacted with DNA. The work done to prevent the damages that may arise from the effects of these effects is being seriously projected.*
Materials and method: *In this study, the oxidative DNA damage inhibitory effect of ethanol extract of almond (A. trichamygdalus) fruits grown in Eğil province of Diyarbakır province in copper presence or absence was investigated. Extract was prepared from dried almond (A. trichamygdalus) fruit collected from Diyarbakır Ergani region using the soxlet method. Horizontal electrophoresis method was used in the study. Photographed with a gel imaging system .*
Result: *It was observed that the almond fruit had a protective effect against the OH radical formed by the H_2O_2 photolysis, that is, the DNA extract inhibited the ethanol extract at a concentration range of 50-1000 µg / ml. Increased concentrations of the ethanol extract of A. trichamygdalus fruit in the presence of copper have been shown to increase DNA cut.*
Key words: *A. Trichamygdalus, Almond, Copper, DNA damage, Cancer*

Date of Submission: 22-02-2018 Date of acceptance: 08-03-2018

I. Introduction

Free radicals are molecular structures that can occur during normal cellular metabolism or due to some external factors (Kopani et al., 2006).

It is known that free radicals are missing electrons in molecular structures and have high energy structures. Because of this state of free radicals, DNA, Lipid, Protein etc. can be transferred into the stable structure. They can cause very serious damages by attacking the construction like. Very serious diseases can occur if free radicals can not prevent these damages. At the beginning of these diseases can come many diseases such as cancer, atherosclerosis, diabetes, alzheimer, aging (Dilas and Canadanovic, 2012).

Examples are oxygen-free free radicals, including superoxide ($O_2^{\bullet}$), Hydroxyl ($^{\bullet}OH$), peroxyl (ROO), alkoxyl (RO), and nitric oxide ($NO^{\bullet}$), and singlet oxygen (10_2), hydrogen peroxide (H_2O_2) and hypochlorite (HOCl) are compounds known as reactive oxygen species (ROT) (Stahl et al., 1993).

The Effects of Free Radicals on DNA Damage

All of the changes that occur in the molecular integrity of the genetic material due to the etiology of endogenous or exogenous factors can be called DNA damage. DNA can be constantly affected by external threats. In the face of cellular events such as DNA replication and recombination, endogenous changes occur constantly in DNA structure. (Kulaksiz et al., 2007).

Because DNA contains a large number of negatively charged phosphate groups, it is an anion that has the ability to bind various cations. Fe^{+2} / $^{+3}$ and Cu^{+1} / $^{+2}$ ions can be found to be permanently bound to negatively charged DNA, and they can bind to DNA by releasing iron and copper proteins in the cell under oxidative stres. The bonds of redox active transition metal ions make the DNA molecule the target of H_2O_2. Hydroxyl radicals (.OH) formed by the reaction of H_2O_2 with DNA with metal ions attached to DNA can not be

removed by hydroxyl radical extinguishers. At the same time, the products they produce from hydroxy radicals cause very serious damage to the DNA structure (Halliwell, 1999).

II. Methodology

The material of this work is Amygdalus trichamygdalus (HAND-Mazz.) Woronow. trichamygdalus Rosaceae c.v. fruit is sweet cultured almonds grown in the province of Diyarbakır - Eğil. The extract was prepared from the dry almond (A. trichamygdalus) fruit collected from the Ergani region of Diyarbakır using the soksalet method to prepare the plant's ethanol extract. As a result of this treatment, 1.015 gram extract was obtained. The resulting extract was stored in a container at + 4 °C.

DNA Agarose Gel Electrophoresis

The experiment was carried out using the horizontal gel electrophoresis method. For the experiment, some unprocessed DNA in some epondroflard, some UV only, or only H_2O_2 added. Plasmid DNA was used as a control. Here we prepared 16 separate wells for gel electrophoresis to see the DNA fragment.

wells	Reactıon Condıtıons
1. well	DNA
2. well	DNA + *A.t* (50 µg/ml),
3. well	DNA + *A.t* (100 µg/ml)
4. well	DNA + *A.t* (250 µg/ml)
5. well	DNA + *A.t* (350 µg/ml)
6. well	DNA + *A.t* (500 µg/ml)
7. well	DNA + *A.t* (750 µg/ml)
8. well	DNA + *A.t* (1000 µg/ml)
9. well	DNA + $CuCl_2$
10. well	DNA + $CuCl_2$ + *A.t* (50 µg/ml)
11. well	DNA + $CuCl_2$ + *A.t* (100 µg/ml)
12. well	DNA + $CuCl_2$ + *A.t* (250 µg/ml)
13. well	DNA + $CuCl_2$ + *A.t* (350 µg/ml)
14. well	DNA + $CuCl_2$ + *A.t* (500 µg/ml)
15. well	DNA + $CuCl_2$ + *A.t* (750 µg/ml)
16. well	DNA + $CuCl_2$ + *A.t* (1000 µg/ml)

Table 1. Reaction conditions in wells in DNA gel electrophoresis copper presence or absence

After the electrophoresis process was finished, the gel was photographed with a gel imaging system. Percent inhibition values were calculated using the following equation.

$$\%I = 1-[(S_{m+a}-S_c)/(S_m-S_c)] \times 100$$

Sc = Percentage of supercoiled form in untreated control DNA, Sm = Percentage of supercoiled form remaining after interaction with the reaction mixture outside the substance that inhibits DNA segmentation, Sm + a = the percentage of supercoiled form remaining after the reaction with the reaction mixture in the presence of the substance believed to inhibit DNA segmentation.

III. Results

In the present study, only some of the wells with plasmid DNA had fruit extracts at different concentrations. Cu^{+2} metal was added to the other wells to investigate the DNA cutting effect of 16 different wel. Here too% of DNA cut% inhibition was calculated using the equation given in the method section above. These values were tried not to be expressed graphically (Figure 1). In this chart it is very clear that A. trichamygdalus ethanol extract inhibits DNA fragmentation according to increasing concentration in the range of 50-1000 mg / ml. In the same graph, when we added A. trichamygdalus together with Cu + 2 ion in increasing concentration in the end result, it was very clear that the DNA was cut off.

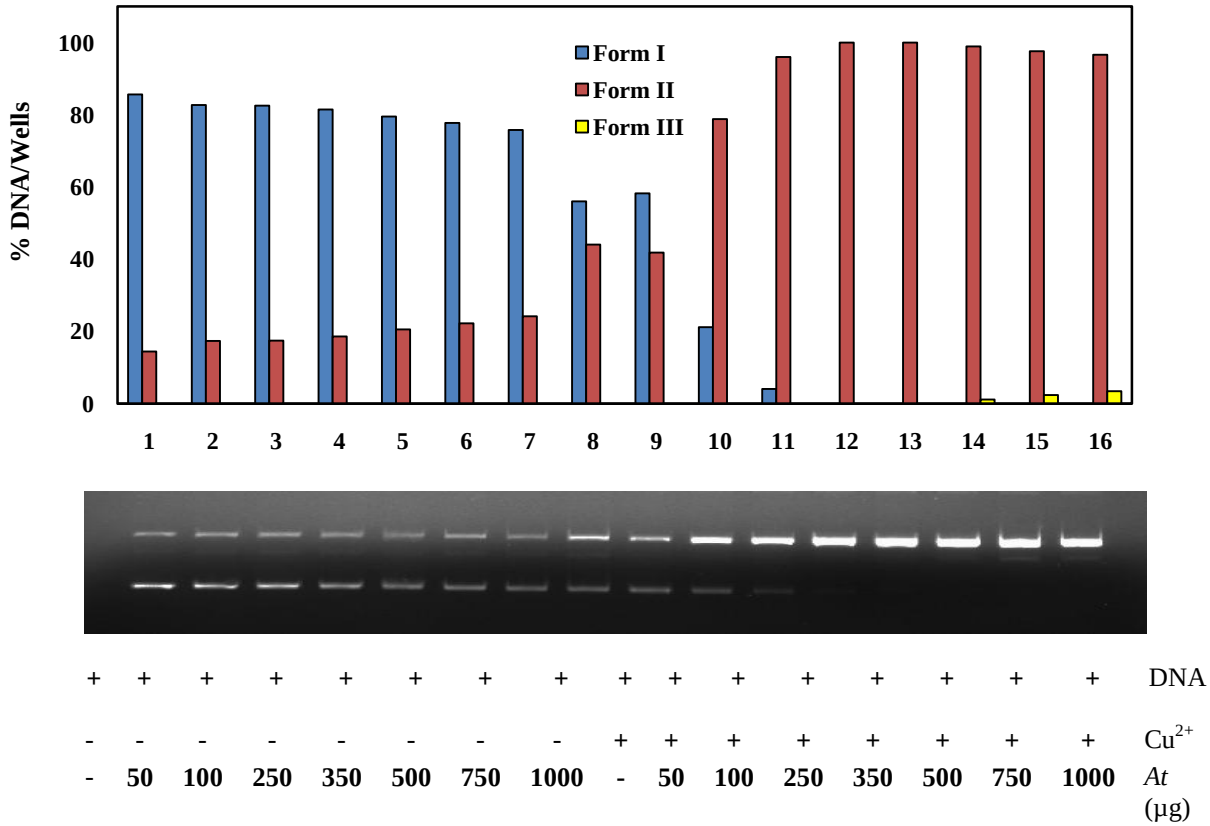

Figure 1. Investigation of the effect of ethanol extract of A. trichamygdalus (A. t) on DNA in the presence and absence of copper. Electrophoresis was performed in a 1% agarose gel containing ethidium bromide (10 mg / mL) for 1 hour at 90 V with 500 mA current. Electrophoresis buffer: TAE (40 mM Tris acetate, 1 mM EDTA, pH 8.2); Jellerin photo Bio Rad Gel Doc XR (BioRad, Hercules, CA, USA). imaging system, and% cut calculations were calculated with the Quantity One program (version 4.5.2, BioRad Co.)

Table 2. Form percentages of the effect of the ethanol extract of A. trichamygdalus juice on DNA fragments in the presence or absence of copper.

		Form (%)		
wells	Reaction	form-1	form-2	form-3
1	DNA	86.17	13.83	
2	DNA + 50 µg/ml *A.t.*	84.48	15.52	
3	DNA + 100 µg/ml *A.t.*	84.12	15.88	
4	DNA + 250 µg/ml *A.t.*	83.65	16.35	
5	DNA + 350 µg/ml *A.t.*	80.11	19.89	
6	DNA + 500 µg/ml *A.t.*	79.67	20.33	
7	DNA + 750 µg/ml *A.t.*	78.33	21.67	
8	DNA + 1000 µg/ml *A.t.*	56.49	43.51	
9	DNA + Cu^{+2}	58.25	41.75	
10	DNA + 50 µg/ml *A.t.* + Cu^{+2}	20.05	79.95	
11	DNA + 100 µg/ml *A.t.* + Cu^{+2}	5.12	94.88	
12	DNA + 250 µg/ml *A.t.* + Cu^{+2}		100	
13	DNA + 350 µg/ml *A.t.* + Cu^{+2}		100	
14	DNA + 500 µg/ml *A.t.* + Cu^{+2}		98.06	1.94
15	DNA + 750 µg/ml *A.t.* + Cu^{+2}		96.98	3.02
16	DNA + 1000 µg/ml *A.t.*+Cu^{+2}		95.45	4.55

IV. Discussion

Wang et al. were investigated the effect of pyridine-containing crown ethers on the Cu^{2+} complex and the effect of this complex on the DNA fragment. The results show that the Cu^{2+} complex hydrolyzes the phosphodiester bond in supercoiled DNA (Wang, 2008).

In a study by Ma and colleagues, it was observed that the methanol-water extract of Commiphora africana cuts DNA in Cu^{2+} presence. The crude extract was fractionated to purify the active compound that caused the DNA cleavage and this compound was named phellamurin (Ma 2005).

V. Conclusion

The study concludes that A. trichamygdalus fruit is a good prooxidant besides its antioxidant properties. This prooxidant property is believed to be due to some components present in the almond fruit. It appears very well that the Cu^{2+} ion enhances the DNA segment through interaction with these components. The active substance causing the DNA fragmentation can be identified and purified. Due to the prooxidant property of A. trichamygdalus fruit, more detailed studies and necessary isolation procedures can be performed. Isolation is very important in that the active substance can be used in the production of anticancer drugs.

VI. Acknowledgement

This study was supported by VAN YYU Scientific Research Projects Project as FDK-2017-5305 project. We would like to thank the Presidency of the Scientific Research Projects for their support.

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IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) is UGC approved Journal with Sl. No. 4033, Journal no. 44202.

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