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**ANNQUEST MAY 2022**

 **ISSN: 2321-3043**

**Effect of β-carotene on denatured human DNA**

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Abstract

Beta-carotene(β) are abundant micronutrients in our diet with antioxidant activity.They protect our body from damaging free radicals,which are the primary cause of aging and degradation.They play a critical role in maintaining healthy vision,skin and neurological functions, and lower the risk of developing cancer and heart diseases.This study tested the effectiveness of β- carotene on renaturation of human DNA, Spinach was chosen due to its abundant levels of β- carotene.β carotene was extracted from spinach using column chromatography technique with dichloromethane as solvent.Theβ-carotene thus extracted was diluted to different concentrations and tested on human DNA, extracted from human cheeks cells.The DNA was left at room temperature for 48 hours for denaturation before testing.The optical density(OD) values were taken at different times of incubation. It was observed that the protective effect of β-carotene extract on human DNA is efficient at 40 minutes on an average,however there is a decrease in OD values suggesting possible DNA repair in one of the samples.

Key words: β-Carotene, DNA damage, Renaturation

Introduction:

* The phytonutrients present in green leafy vegetables give many common health benefits as it improves nutritional status and reduces risk of specific diseases like diabetes,cancer, and hepatotoxicity.
* β-carotene is an orange-red organic pigment that is abundant in plants and fruits[5].It belongs to a carotene, which is terpenoids (isoprenoids), which are biochemically synthesized from eight isoprene units and therefore have 40 carbons. Among the carotenes, β-carotene is distinguished by the fact that it has beta rings at both ends. Beta-carotene is biosynthesized from geranylgeranyl pyrophosphate [6].
* Plant carotenoids are the primary dietary source of provitamin A worldwide, with β carotene having the best-known pro vitamin A carotenoid.
* One molecule of β carotene can be cleaved by the intestinal enzyme β, β-carotene 15 15'-monooxygenase into 2 molecules of vitamin A.

β-carotene not only plays an important role in providing vitamin A but also determines whether β carotene should be converted into vitamin A or should be circulated in the form of β carotene. Vitamin A also plays an important role in immune function and cellular communication [7].It protects your skin and eyes, and fight, life-threatening conditions like heart disease and cancer.β carotene may have a diverse effect when taken in high doses by people who smoke or who have been exposed to asbestos.It’s clear that foods containing beta carotene and other antioxidant helps lower levels of inflammation and fights oxidative stress within the body.

Spinach (Spinacia oleracea) is an annual edible flowering plant from the Amaranthaceae family. It is a green, leafy vegetable[1] and it comes from southwestern and central Asia.It is an annual plant.Spinach can survive winter in temperate regions[2]. The leaves are alternate,simple,ovate to triangular in shape and vary in size from long to broad with larger leaves at the base of the plant and small leaves higher on the flowering stem(the flowers are small and yellow)green,maturing into a small,hard,dry, lumpy fruit,luster,containing several seeds[3]. Spinach contains oxalic acidswhich causes a lingering bitter taste. It is rich in vitamins and beta carotene and has strong antioxidant effects.

Spinaciaoleracea has been used since ancient periods as a vitamin’s rich food source.It contains many nutrients and minerals which have high amounts of vitamins A,K and the vitamin B folate that promotes normal fetal development during pregnancy.Spinach provides a dual protection against oxidative DNA damage, enhancing antioxidant leaves and stimulating DNA repair,that may be beneficial in neurological functions.Spinach is also high in fiber and water, both of which help with constipation and promote a healthy digestive tract[4]. The risk of developing asthma is lower in people who consume a high amount of spinach.

Materials and methods:

* β-carotene extractions from spinach leaves:

10g of spinach leaves were treated with 30ml of ethanol to remove the water in the leaves. The sample was then taken out and pressed dry on a paper towel to remove all feces of water and ethanol. Following which, 40ml of dichloromethane was added and the leaves were ground to extract chlorophyll and beta carotene.This mixture was then filtered to remove the leaves and the resultant filtrate was heated until about 2ml remained.

* Column chromatography:

The chromatography column was assembled by placing a small cotton plug in the bottom of the column,followed by pouring around 2 mm of sea sand over the plug.This prevented the solid alumina from entering the stop lock. Hexane was added to a log of alumina to produce a slurry that was added on top of the sea sand. Excess hexane was drained off until only 0.5 cm of hexane covered the aluminum oxide. 2 ml of the plant extract was carefully transferred to the column. Hexane was added and the eluted yellow beta-carotene was collected in a dry test tube.[8].

* Extraction of human DNA from cheek cells:

500 ml of drinking water was mixed with 1 tablespoon of salt and stirred until the salt dissolved. Then 3 tablespoons of salt water were used to gargle for a minute and then pit into a cup. A drop of soap was then added into the cup and gently stirred (Avoid bubbles if possible). In a separate cup, 100 ml of isopropyl alcohol was mixed with three drops of food coloring. This mixture of alcoholand food coloring was mixed into the cup containing the salt water which was used to gargle and set aside for 25minutes. Alcohol was added to help isolate DNA in (about 2cm thick) one forms a layer on top of the mixture. DNA was seen in white clumps and threads.[9]

* The β- carotene extract with variable concentrations was then exposed to human DNA samples at different time intervals. (Table No-1)



Table- 1

Results and discussion:

OD values of treated DNA samples taken at 440nm using calorimetry revealed a variation in different concentrations of β- carotene diluted with water.The decrease in OD values overtime may indicate the repair of DNA.

There is no consistency in results reported, however, the following trends have been observed in different samples.

Sample 1. There is a decrease in OD values in the time duration between 10-70 minutes.

Sample 2. It shows fluctuations of increase followed by decrease and increase in different time periods.

Sample 3. The trends are similar to sample 2

Sample 4.There are marginal fluctuations among time periods

Sample 5. Fluctuations are similar to sample 2& 3

Reasons could be:

* These samples were incubated at room temperature in different tubes;DNA damage might have occurred in random.
* The repair may be proportional to the amount of DNA damage in each sample

Conclusion

From sample 1, one can infer that the required incubation time for DNA repair is between 10-70 minutes duration.

However, further investigations are needed to clearly evaluate the effects of Beta carotene.

Acknowledgements: The authors acknowledge the support and encouragement given by Principal and Management of the college.

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