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PROTECTION OF DNA FROM OXIDATIVE DAMAGE OF H_2O_2 BY CITRUS X SINENSIS PEEL POWDER

Dr.B.Eswari
Assistant Professor
Department of Chemical sciences,
Sree Vidyanikethan Degree College, A.P, India.

D.Harika
Department of Chemical Sciences
Sree Vidyanikethan Degree College, A.P, India

Abstract - **Consumption of natural antioxidants through diet is necessary as the free radicals generated in the body should be eliminated from the biological system for the proper functioning of the system. Vit A, E and C are the natural antioxidants that protect biomolecules from the oxidative damage. In this study we used H2O2 to induce oxidative damage and C. sinensis peel powder as the protective agent against DNA Damage. 20% and 30% H2O2 caused extensive damage to the DNA double strand compared to 10% H2O2 in dose dependent manner. Oxidative damage was recovered up to 90% with C. sinensis peel powder in case of salmon milt DNA but the protection was negligible in case of DNA isolated from chicken liver.**

Key words: **C. sinensis, H2O2, Oxidative damage**

I. INTRODUCTION

Oxidative stress and oxidative damage are the exaggerated responses of the living system due to increased oxidative insult caused by free radicals produced inside the biological system and accidental ingestion. Lipid peroxidation caused by free radicals results in membrane collapse due to presence of unsaturated lipids in the membrane barrier. Proteins are also susceptible due to presence of aminoacid cysteine in them. DNA is highly susceptible to damage as the purines and pyrimidines contain double bonds.

DNA base adducts and allyl radicals are generated in response to exposed oxidants and in case of thymine H is withdrawn from the methyl group. OH radicals are the more common generated adducts in DNA. Both the abstraction of H atom and generated OH radicals at C5 and
C6 leads to generation of allyl radical and the radical is oxidising in case of C6 OH[(1)].

Orange peel consists of biochemical components like cellulose, hemi cellulose, lignin and low amounts of limonene. The peel serves as a good antioxidant, has anticarcinogenic effects and proven to be effective against breast and colon cancer, muscle pain and ring worm infections [(2-4)]. Orange Peel also possess anti microbial activity and found to be effective against both Gram positive and gram negative bacteria like Staphylococcus aureus, Listeria monocytogens and Pseudomonas aeruginosa [(5)].

Maltease Citrus peel consists of soluble carbohydrates, proteins, phenols which are majorly responsible for antioxidant activity and glycosylated flavanones and polymethoxylated flavones [(6)].73%-97% of essential oils of peel contains d-limonene as from Geraci etal., 2017 [(7)]. Protection of DNA cleavage by orange peel (Citrus sinensis) from H2O2 was first reported from our lab and proven to be effective against salmon milt DNA compared to Gallus gallus domesticus DNA isolated from liver.

II. RESULTS

A. Analysis of oxidative damage on DNA Treated with H2O2:

From the figure 1 oxidative damage induced by H2O2 produced short stretches of DNA and the damage was extensive with 20% and 30% H2O2 in dose dependent manner, due to they migrated faster compared to the DNA treated with 10% H2O2 and not visual in the gel.

B. Assessment of Protective effect of C.sinensis peel aqueous extract on oxidative damage induced by H2O2

From the figure 2 DNA isolated from the chicken liver and protein content was higher compared to DNA observed by photometric determination of DNA at 260nm and 280nm. The ratio of A260/280 is

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1.102. and the damage of DNA isolated from chicken liver was extensive with 20% and 30% H2O2 compared to salmon milt DNA and hence difficult to capture the damage using AGE.

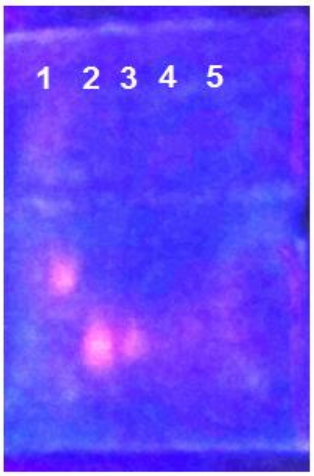

Figure: 1 Electrophoresis of DNA treated with H2O2.Well 1- Molecular marker, Well 2- control, Well 3,4,5 – DNA treated with 10%,20% and 30% H2O2.

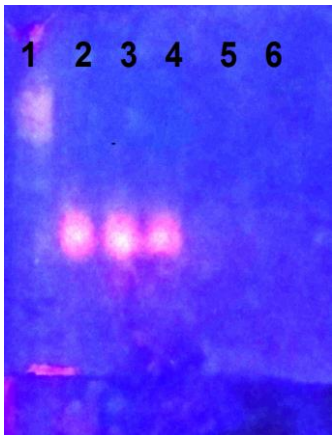

Figure :2 DNA Treated with H2O2 and C. sinensis peel powder aqueous extract. Well 1- molecular marker, well 2 – control, well 3,well4 – commercially available DNA treated with 20% and 30% H2O2 and10% plant extract, well5&6-Biological sample treated with 20% and 30% H2O2 and 10% plant extract .

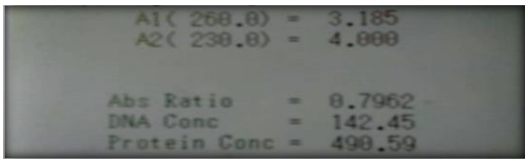

Figure: 3 DNA Quantification using spectrophotometric method. The Purity of DNA was calibrated by A260/280 value.

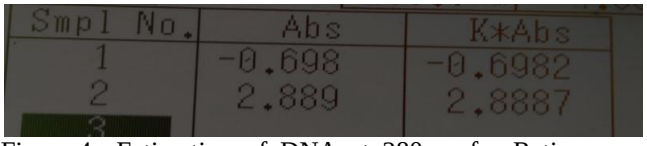

Figure:4 Estimation of DNA at 280nm for Ratio. Sample 1 is the absorbance before base correction (Auto Zero), Sample 2 is the sample of DNA from chicken liver.

Due to high compactness of DNA isolated from chicken liver, it is highly prone to damage than of the salmon milt DNA available commercially. C.sinensis contains vit C in high amounts and can confer protection to oxidative damage and due to high concentration of DNA in case of biological sample 10% solution of citrus sinensis peel aqueous extract is not efficient for protecting against oxidative damage.

III. METHODS

10g of Citrus sinesis peel was weighed and transferred to 100ml of water, stirred for 15 min on magnetic stirrer and the resultant extract was filtered through muslin cloth and used as plant extract.

Analysis of Oxidative Damage on DNA treated with H2O2:

Oxidative damage of DNA by H2O2 was assayed by agarose gel electrophoresis explained below. Commercially available salmon milt DNA was used at a concentration of 1mg/ml and 200µl each is transferred in to eppendof tubes and treated with 100µl H2O2 for 30 min. After the time period 100µl of above plant extract was added to each eppendorf tube and treated for 30min. After the time period the samples were run on 1% agarose gel and visualised under UV chamber. Untreated DNA was taken as positive control.

IV. DISCUSSION

H2O2 is the key modulator of oxidative stress in oxygen generated free radicals along with superoxides. From Subhashinee S K Wijeratne etal (2005) H2O2 is involved in oxidative damage of CaCo-2 colon cancer cells [8] due to high percent of DNA damage and cell membrane burst.

According to Helmut Sies (2017) [9] H2o2 is involved in activation of HIF which is responsible for

angiogenesis in colon cancer cells with less damage to cell membrane as catalase is mainly localised in cell membrane and hence the damage is restricted to DNA but stimulates the growth of new cancer cells through HIF at low doses. DNA damage is not repaired in cancer cells hence they are more susceptible to H2O2 damage compared to normal cells.

According to Henzler T et al., 2000 & Bienert G.P et al., 2007 H2O2 can cross the aquaporins and hence known as peroxiporins. They can modulate several transcription factors in mammalian cells and the major TFs they target include HIF,NF-KB, PTEN, Notch and contribute to redox signalling in cells through peroxiredoxins[10,11]. H2O2 can target several biological functions such as apoptosis, Muscle contraction, circadian rhytms, Proliferation [12,13,14].

From Herzog etal., 2016 Endoplasmic reticulum act as a modulator of H2O2 signaling through aquaporins and peroxiredoxins [15,16,17]. Cancer cells have high level of Aquaporins hence we can target tumor cells with treatment of H2O2 as they have low repair capacity than normal proliferating cells.

Redox level balance in cell is maintained by catalase, SOD and Glutathione peroxidise through neutralising free radicals generated in the system. Sulfenic acids an anolog of H2O2 can protect against oxidants, also has role in signalling form major basis of research now according to Poole LB et al., 2013 [18]. Hence H2O2 promotes cell proliferation and growth at low amounts and causes oxidative damage and death due to damage in DNA and cell membrane.

Citrus X sinensis has high vit C which can serve as antioxidant can protect the DNA damage in normal cells compared to cancer cells at low concentrations of H2O2.

V. CONCLUSION

C.sinensis has high concentration of antioxidant Vit-C and it can effectively repair the damage induced in the salmon milt DNA but due to high concentration of DNA isolated from biological sample may be the damage is extensive and the protective effect is not seen.

VI. REFERENCES

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