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PROTECTION OF DNA FROM OXIDATIVE DAMAGE OF H₂O₂ BY CITRUS X SINENSIS PEEL POWDER

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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 amino acid 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DNA base adducts and allyl radicals are generated in response to exposed oxidants and in case of thymine H is withdrawn from the methyl group.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, hemicellulose, 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⁾.

Maltese 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 et al., 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 visible 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

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.

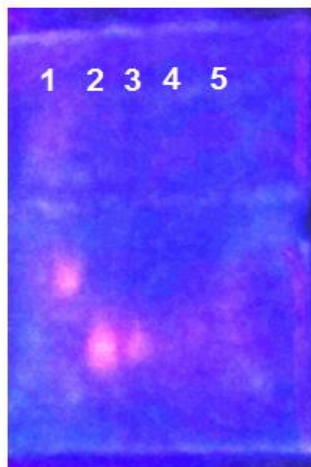


Figure: 1 Electrophoresis of DNA treated with H₂O₂. Well 1- Molecular marker, Well 2- control, Well 3,4,5 – DNA treated with 10%,20% and 30% H₂O₂.

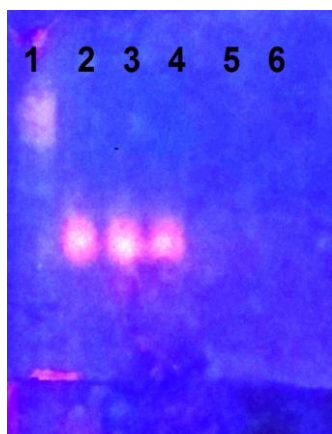


Figure :2 DNA Treated with H₂O₂ and C. sinensis peel powder aqueous extract. Well 1- molecular marker, well 2 – control, well 3,well4 – commercially available DNA treated with 20% and 30% H₂O₂ and 10% plant extract, well 5&6-Biological sample treated with 20% and 30% H₂O₂ and 10% plant extract .

A1(260.8) =	3.185
A2(230.8) =	4.000
Abs Ratio =	0.7962
DNA Conc =	142.45
Protein Conc =	490.59

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

Smpl No.	Abs	K*Abs
1	-0.698	-0.6982
2	2.889	2.8887

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 sinensis 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 eppendorf 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 et al (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- κ B, 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 rhythms, Proliferation^(12,13,14).

From Herzog et al., 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 peroxidase through neutralising free radicals generated in the system. Sulfenic acids an analog 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

1. Marcus Cooke S, Mark Evans D., Miral D., Joseph Lunec (2003). Oxidative DNA damage: mechanisms, mutation, and disease. FASEB, (pp1195-1214).
2. Midha, S (2012). Horticulture Statistics in India, Horticulture Statistics in India, Report. (pp 148–161).
3. Palma, C., Contreras E., Urra, J. and Martinez M.J (2011). Ecofriendly technologies based on banana peel use for the decolourization of the dyeing process wastewater. Waste Biomass Valor. (pp 77–86).
4. Wadhwa, M., and Bakshi, M. P. S (2013). Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products. FAO (UN) RAP Publication (pp 1-46).
5. Geraci Anna & Di Stefano, Vita & Di Martino, Enrica & Schillaci, Domenico & Schicchi, Rosario. (2016). Essential oil components of orange peels and antimicrobial activity. Natural product research. (pp1-7).
6. M'hiri N., Ioannou I., Ghoul M., Mihoubi Boudhrioua N., (2015). Proximate chemical composition of orange peel and variation of phenols and antioxidant activity during convective air drying. Journal of new sciences, Agriculture and Biotechnology, JS-INAT. (pp 881-890).
7. Anna Geraci, Vita Di Stefano, Enrica Di Martino, Domenico Schillaci, Rosario Schicchi (2017). Essential oil components of orange peels and antimicrobial activity Nat Prod Res (pp 653-659). PMID: 27535797
8. Subhashinee Wijeratne S K., Susan Cuppett L., Vicky Schlegel (2005). Hydrogen peroxide induced oxidative stress damage and antioxidant enzyme response in Caco-2 human colon cells J Agric Food Chem (pp 8768-74).
9. Helmut Sies (2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative stress. Redox Biol. (pp 613–619). PMID: 28110218
10. Sobotta M.C., Liou W., Stocker S., Talwar D., Oehler M (2015). Peroxiredoxin-2 and STAT3 form a redox relay for H₂O₂ signaling. Nat. Chem. Biol. (pp64–70). [PubMed] [Google Scholar]
11. Rhee S.G., Woo H.A (2011). Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger H₂O₂, and protein chaperones. Antioxid. Redox Signal. (pp 781–794). [PubMed] [Google Scholar]
12. Henzler T., Steudle E (2000). Transport and metabolic degradation of hydrogen peroxide in Chara corallina: model calculations and



- measurements with the pressure probe suggest transport of H₂O₂ across water channels. *J. Exp. Bot.* (pp 2053–2066). [PubMed] [Google Scholar]
13. Bienert G.P., Schjoerring J.K., Jahn T.P (2006). Membrane transport of hydrogen peroxide. *Biochim. Biophys. Acta.* ; (pp 994–1003). [PubMed] [Google Scholar]
 14. Bienert G.P., Moller A.L., Kristiansen K.A., Schulz A., Moller I.M (2007). Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *J. Biol. Chem.* ; (pp 1183–1192). [PubMed] [Google Scholar]
 15. Marios C. Papadopoulos Samira Saadoun (2015). Key roles of aquaporins in tumor biology *Biochimica et Biophysica Acta* (pp 2576–2583).
 16. Appenzeller-Herzog C., Banhegyi G., Bogeski I., Davies K.J., Delaunay-Moisan A (2016). Transit of HO across the endoplasmic reticulum membrane is not sluggish. *Free Radic. Biol. Med.* (pp 157–160). [PubMed] [Google Scholar]
 17. Katarzyna K and Krzysztof S (2019). DNA repair in cancer initiation, progression, and therapy—a double-edged sword. *J Appl Genet.* (pp 329–334). PMID: PMC6803590, PMID: 31468363
 18. Poole LB, Nelson KJ, Karplus PA (2013). Sulfenic acids and peroxiredoxins in oxidant defense and signaling. In: Jakob U, Reichmann D, editors. *Oxidative stress and redox regulation*. Dordrecht: Springer: (pp 85–118).

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