Docking Exploration of Human Estrogen Receptor to Decipher Phytochemicals as Tumor Suppressors

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Abstract:- **The most common type of cancer in women all over the world is breast cancer. The estrogen molecule is the major risk factor related to mammary gland tumors. Phytochemicals block the action of carcinogens on the target cells by binding to specific receptors. The aim of the present study is to evaluate the binding affinity and interactions of phytochemicals like Anthocyanin, Isoflavones and Carnosol with Human Estrogen receptor (hER) as tumor suppressors. Discovery Studio 3.1 and Auto Dock 1.5.6 were used to dock phytochemicals with hER. Docking analysis of Anthocyanin and Isoflavones to hER showed that Lys 449, Pro 324, Gly 390, Arg 394, Trp 393 and Glu353are the potential drug targets. Carnosol displayedadditional interactions at Ile 326, Leu 387 and Met 357 in the active site of hER. Carnosol exhibited higher binding affinity (12.1) than Anthocyanins and Isoflavones (8.9). The present study suggests that Carnosol, Anthocyanins and Isoflavones can be used as potent drug in preventing breast cancer.Risk of Breast cancer in women can be reduced significantly by consuming plant foods rich in phytochemicals like Carnosol, Anthocyanin and Isoflavones.**

Key words: Anthocyanin, Isoflavones, Carnosol, Human Estrogen receptor, Mammary gland tumour, Binding affinity, Breast cancer.

1. INTRODUCTION

Breast Cancer, with frequency of 10.4 % percent is the second mostpredominant type of cancer after lung cancer (WHO, 2006). Biochemical and immunohistochemical techniques of Mulas, *et al*., (2001 and 2005) and Illera, *et al*., (2006) suggestedthat ER-αand ER-β were involved in benign and malignant mammary gland tumor. Both normalbreast cells and most breast cancer cells have receptors to bind blood estrogen and progesterone (Mark, *et al*., 2010). These hormones bind to the receptors and cause cell proliferation and growth through signal cascade (Saba, 2013). Estrogen and progesterone function with oncogenes and tumorsuppressor genes causing the cell to grow out of control (Caroline, 2003).Chemical drugs treating breast cancer causes varied side effects likeleg pain, trouble in breathing, chest pain and vision changesthat makes these drugs unsuitable for treatment and need for a better alternate. Phytochemicals are proved to be very successful to diminish the possibility of cancer (Saba, 2013). Phytochemicals were classified into many groups (Herman, 2002, Aedin, *et al*., 2007, Maria, *et al*., 2010) chiefly Flavonoids andIsoflavones. The structure of Isoflavones is analogous to estrogen and it compete with estrogen for the same receptor sites thereby blocking estrogen, a hormone linked to an increased risk of breast and other hormone-dependent cancers (Kenneth, *et al.*, 1999). Anthocyanins are water soluble flavonoids with vacuolar pigments that occur naturally in fruits and vegetables as glycosides. Carnosol is one of the polyphenolic antioxidants and anticarcinogen found in extracts of the herb rosemary (*Rosmarinus officinalis*) (Lo, *et al*., 2011). Docking predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Lengauer, *et al*., 1996, Alex and Nixon., 2009).Docking of anthocyanin, isoflavones and carnosol into the binding site of a human estrogen receptor reveal the binding affinity of the complex which is a key part in structure-based drug designing (Daniel, *et al*., 2010). The present investigation aims to determine the interaction pattern of anthocyanin, isoflavones and carnosol with human estrogen receptor using AutoDock Tool 1.5.6.

2. EXPERIMENTAL

Biological databases like PubChem, Drug Bank, PDB (Protein Data Bank) and software's like Open Babel, Discovery Studio 3.1 and AutoDock 1.56 were used for the present study.

2.1. Generation of 3D structures

The Protein Data Bank (PDB; http://www.rcsb.org/pdb/) is the worldwide archive of structural data of biological macromolecules. The structure of human estrogen receptor was retrieved from PDB (Berman,*et al*., 2002, Berman, *et al.*, 2006).

2.1.1 Structure of phytochemicals

Structure for anthocyanin (CID 145858), isoflavone (CID 72304) and carnosol (442009) were retrieved from NCBI Pubchem compound. The retrieved structures were validated and all the hetero atoms were removed for efficient molecular docking studies (Wishart,*et al.*, 2008, Wishart *et al*., 2000).

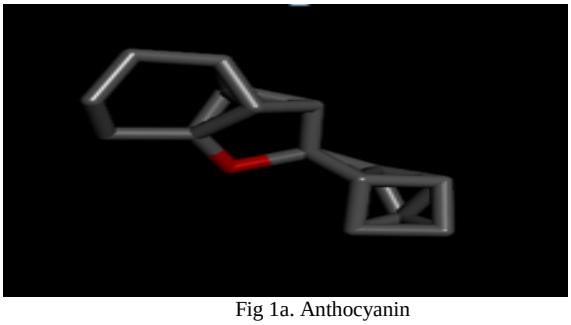

Fig 1a. Anthocyanin

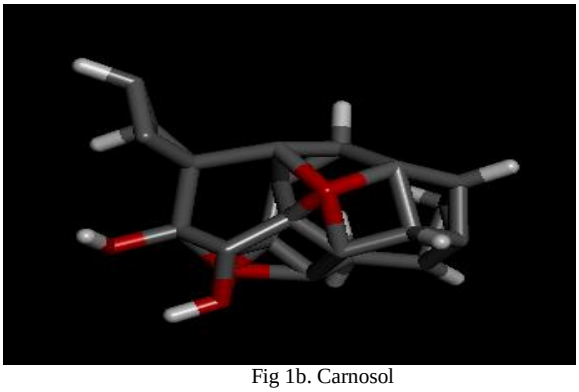

Fig 1b. Carnosol

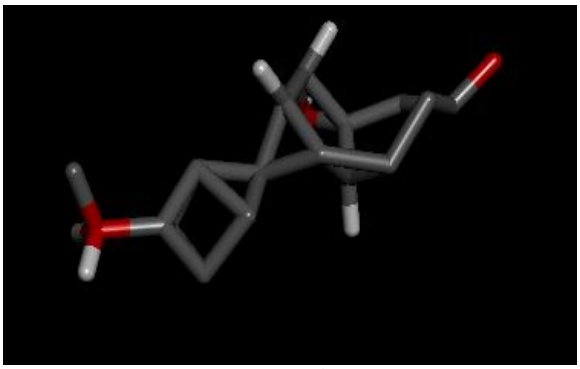

Fig 1c. Isoflavone

2.2. Molecular docking

Discovery studio3.1 was used to simulateligands and with human estrogen receptor. The docking analysis of phytochemicals with human estrogen receptor were carried out by AutoDock software that explore ways in which drug and receptor fit together and docks to each other. An extended PDB format, termed as PDBQT file was used to coordinate files which include atomic partial charges. Open Babel was used for creating PDBQT files from traditional PDB files. Autodock1.5.6 was used to predict the binding of ligands to 3D structure of estrogen receptor. Atomic affinity grid was used todesign better binders. In the present study, grid box was set to 60x60x60 A^{o}.

3. RESULTS AND DISCUSSION

3.1. Preparation of human estrogen receptor(hER)

Discovery Studio 3.1 was used to visualize the retrieved 3D structure of hER from PDB, and toidentify the interacting amino acid residues in the active site of the receptor that showed the prevalence of right handed helix. Fig 2 depicts the structure of the after removal of Native ligand, Water molecules, B chains and C chains. Fig 3a, 3b and 3c show the grid box for thehER that were designed using the center values of the ligand.

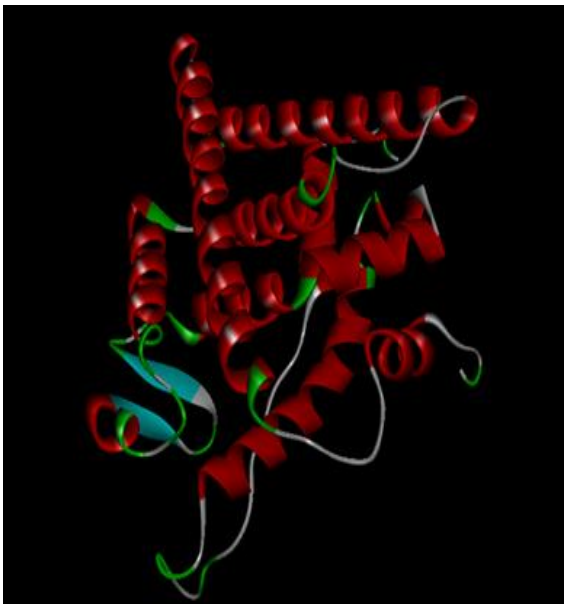

Fig 2. Human Estrogen receptor

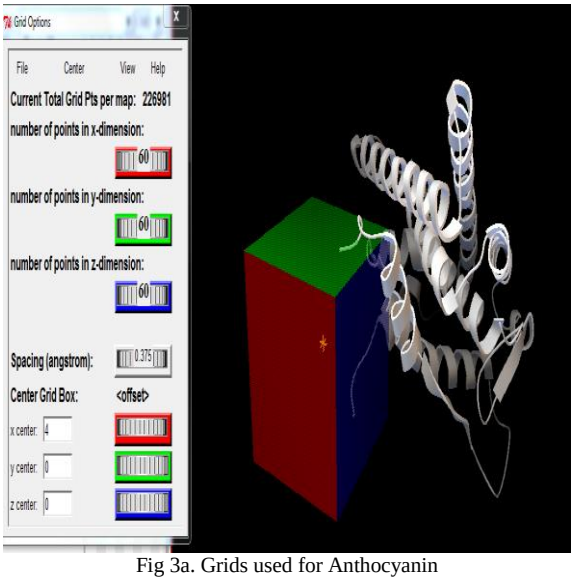

Fig 3a. Grids used for Anthocyanin

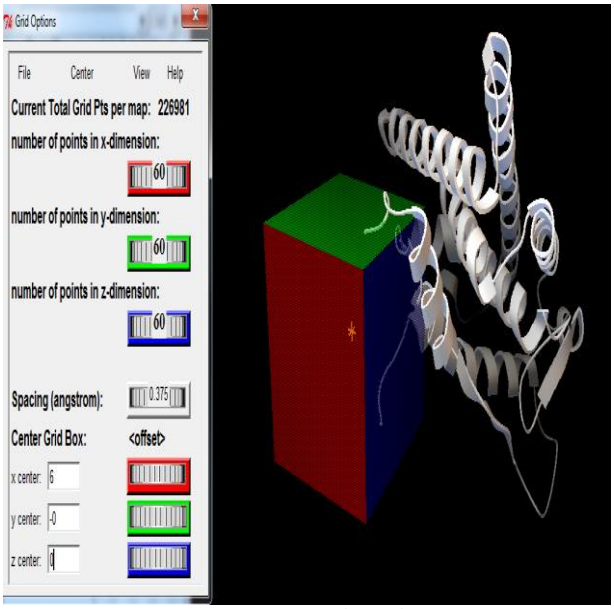

Fig 3b. Grids used for Isoflavones

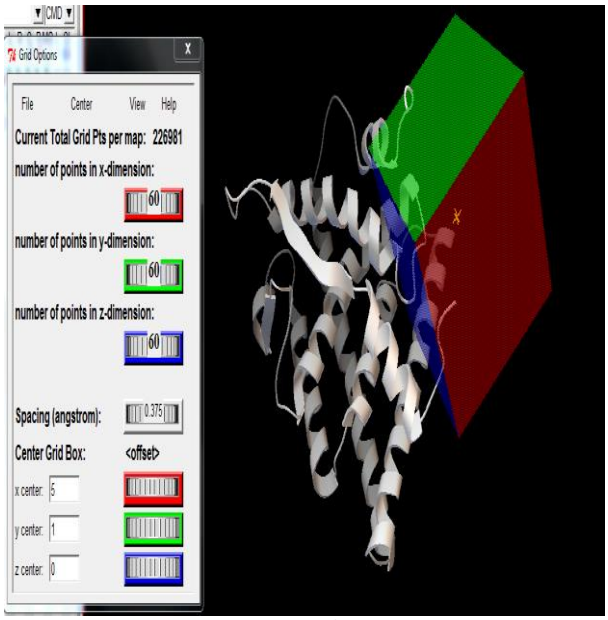

Fig 3c. Grids used for Carnosol.

3.2. Preparation of Ligand

Retrieved molecular structures of the ligands like Anthocyanin, Isoflavone and Carnosol from PubChem in sdf format were and converted to pdb using Open Babel software. The center values (X, Y and Z axis) were visualized using Discovery Studio and were used to set grids for hER.The X, Y and Z values for anthocyanin, Isoflavones and Carnosol (center values) were found to be 4, 0, 0; 6, 0, 0 and 5, 1, 0 respectively.

3.3. Docking Analysis:

AutoDock Tools 1.5.6 was used to convert hER to ribbon style in pdbqt format. The ligands were also converted to pdbqt using Open Babel. Conformation text and command were used to analyze the docked results. The binding affinity of anthocyanin and Isoflavone were found to be 8.9 and Carnosol recorded highest binding affinity (12.1) than other ligands. Saba, *et al*., (2013) also observed that flavonoids have the better binding affinity with Human estrogen receptor due to increased number of intermolecular interactions. In this present study Carnosol showed higher binding affinity with hER due to many number of interactions in the docked complexes than other phytochemicals. This leads to the efficient binding of Carnosol with Human estrogen receptor. The active site amino acids, number of hydrogen bonds and other interactions were determined by AutoDock Tools. Fig. 4a,4b and 4c shows the binding affinities of the ligands and the interacting amino acids in active sites of hER. Ligand binding sites of phytochemicals anthocyanins and isoflavones to hER located by Discovery Studio 3.1 were found to be Lys 449, Pro 324, Gly 390, Arg 394, Trp 393 and Glu353 whereas Carnosol showed additional interactions with active site at Ile 326, Met 357 and Leu 387. The docking study confirmed that the Lys 449, Pro 324, Gly 390, Arg 394, Trp 393 and Glu353 as the potential drug targets. Dykstra, et al., (2007) also reported that Leu 387 serve as one of the catalytic residues inthe three dimensional structure of Human estrogen receptorduring computational analysis of 2-Aryl indoles with hER alpha.

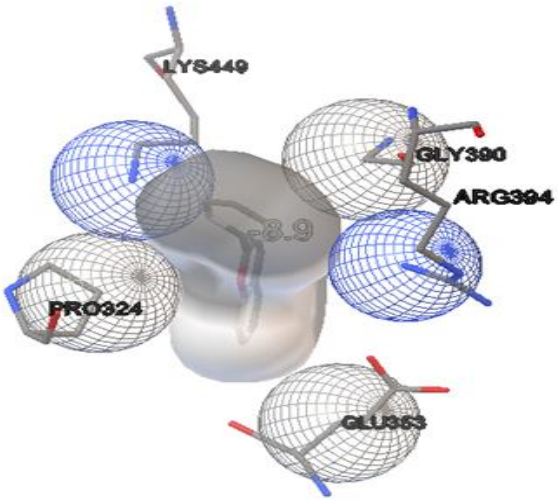

Fig.4a. Anthocyanin with hER

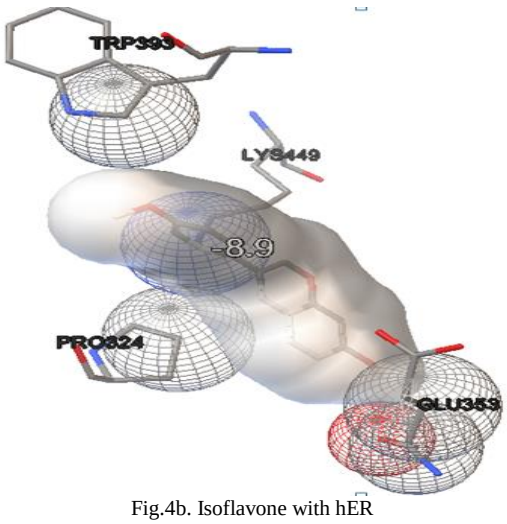

Fig.4b. Isoflavone with hER

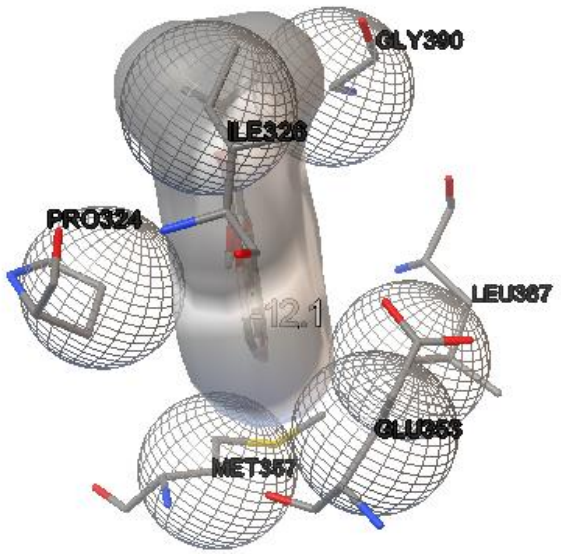

Fig.4c. Carnosol with hER

4. CONCLUSION

Interaction pattern of a phytochemicals to human estrogen receptor were explored by docking analysis using AutoDock Tool 1.5.6.Docked poses of Anthocyanin and Isoflavone showed similar binding affinity of 8.9 to the human estrogen receptor whereas Carnosol depicts higher binding affinity of 12.1. Docked results exhibited that Lys 449, Pro 324, Gly 390, Arg 394, Trp 393, Glu353, Ile 326, Leu 387 and Met 357 were the interacting sites inhER. The present investigation suggests that Carnosol withhigher binding affinity can play a promising role in preventing breast cancer. The phytochemicals can be used as a safe, effective and efficient alternative to chemical drugs in preventing and curing breast cancer.

5. REFERENCES

1. Aedin,C., Bryan,H and Rosa,M (2000). Isoflavones and lignans as potential importance to human health. *Journal of the Science of Food and Agriculture*, 80(7): 1044-1062.
2. Alex. M and Nixon. R (2009). Docking Studies on Anticancer Drugs for Breast Cancer using Hex. *Proceedings of the International MultiConference of Engineers and Computer Scientists*, 2:18 - 20.
3. Berman, H. M., Battistuz, T., Bhat, T. N., Bluhm. W.F., Bourne, P. E (2002). *The protein data bank.Acta Crystallography D. Biol. Crystallogr*, 58: 899-907.
4. Berman H.M, Westbrook. J, Feng. Z, Gilliland, G and Bhat, T. N (2006). *The protein data bank*. 34: 668-672.
5. Daniel, S and Bert, L.,(2010). Ligand docking & binding site analysis with PyMOL and AutoDock/vina.. *Journal of Computer Aided Molecular Design*. 24: 417-422.
6. Dykstra, K.D *et al.*, (2007). Estrogen receptor ligands, Part 16: 2-Aryl indoles as highly subtype selective ligands for ER alpha. *Bioorg. Med. Chem. Lett.*, 17(8):2322–2328.
7. Gianni, L., Corden, B., and Myers, C (1983). The Biochemical Basis of anthracycline Toxicity and Antitumor Activity. *Reviews in Biochemical Toxicology*, 1-82.
8. Herman, A (2002). Phytoestrogens and breast cancer. *The Journal of steroid biochemistry and molecular biology*, 83(1): 113-118.
9. Illera, J.C, Perez-Alenza, M.D and Nieto, A (2006). Steroids and receptors in canine mammary cancer. *Steroids*, 71, 541-8.
10. Kenneth, D.R, Setchell and Aedin, C (1999). Dietry isoflavones: Biological effects and relevance to human health. *The Journal of nutrition*, 129 (3): 758S-767S.
11. Lo, A.H., Liang, Y.C, Lin-Shiau, S.Y., Ho, C.T and Lin, J.K(2012). Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor-kappaB in mouse macrophages.*Carcinogenesis*, 23 (6): 983-991
12. Lengauer, T and Rarey, M (1996). Computational methods for bimolecular docking. *Curr Opin Struct Biol*, 6 (3): 402-406.
13. Maria, R., Carmela, S., Idolo, T and Gian, L.R (2010). Phytochemicals in Cancer prevention and therapy: Truth or dare? *Toxins*, 2(4): 517-551.
14. Mulas, D.L.M.J., Van, N.M and Millana, Y (2000). Immunohistochemical analysis of estrogen receptors in feline mammary gland benign and malignant lesions: comparison with biochemical assay. *Domes Anim Endocr*, 18, 111-25.
15. Mulas, D., Millan, Y and Dios, R (2005). A prospective analysis of Immunohistochemically determined estrogen receptor α and progesterone receptor expression and host and tumor factors as predictors of disease-free period in mammary tumors of the dog. *Vet Pathol*, 42, 200-212.
16. Nelson,L.R andBulun, S.E (2001). Estrogen production and action. Journal of the American Academy of Dermatology; 45(3); 116–124.
17. Saba, F., Muhammad, U. M., Usman, S., (2013). Docking Studies reveal Phytochemicals as the long searched anticancer drugs for breast cancer. *International Journal of Computer Applications*, 67 (25): 1-5.
18. Wishart, D.S, Knox, C., Guo, A.C., Cheng, D and Shrivastava, S (2008). DrugBank: A knowledge base for drugs, drug actions and targets.*Nucleic Acids Res*, 36: 901-906.
19. Wishart, D.S., KnoxGuo, A.C., Shrivastava,S and Hassanaali, M (2000). DrugBank: A Comprehensive resource for *Insilico* drug discovery and exploration. *Nucleic Acids Res*, 28: 235-242.
20. World Health Organization (2006), Fact sheet, No 297: *Cancer*.