Anticancer property of green material through computational approach

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Abstracts

The purpose of research: Colorectal cancer is third most prevalent cancer in developed countries with increasing cases in developing countries. Studies show that the chronic intestinal inflammation is associated with increased risk of developing colorectal cancer. Inflammation is an immunological response to external damaging stimuli and is govern by an endogenous pyrogen and pleiotropic pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α). TNF-α plays an important role in the development of humoral immune response. Production of TNF-α has been implicated in various other pathologies including diabetes, osteoporosis, multiple sclerosis and inflammatory bowel diseases also. Several studies have shown that anti-inflammatory effect of stigmasterol, a phytosterol of an endangered medicinal plant *Chlorophytum borivilianum*, is mediated by suppression of TNF-α. The latter is synthesized as a membrane-anchored precursor. The soluble form of TNF-α is released into extracellular space by tumor necrosis factor alpha converting enzyme (TACE), a multidomain metalloproteinase.

Principal results: We have investigated the anti-cancer effect of the green material against colon cancer by using computational molecular docking and molecular dynamics simulations approach. With this study, we tried to explore stigmasterol as a potential inhibitor of TACE. The active cleft of TACE has a catalytic zinc residue at its centerpentacoordinate by the three-imidazole N_2 atoms of His 405, His 409, His 415. Along with these residues Glu 406, Met 345, Pro 437, Trp 312, Asp 344 around the zinc atom constitute the active site. The docked complex formed a hydrogen bond with Gly 346 and showing hydrophobic interactions with other active site residues. Molecular dynamics simulations confirmed that hydrogen-bond connectivity has not been lost throughout our simulations of 30ns. Computed RSMD suggests that docked complex is stable.

Major conclusions: Therapeutic blockade of TACE could be highly beneficial in case of chronic inflammatory conditions including colon cancer. In the present study, we conclude that stigmasterol, a green material may be considered as a possible therapeutic agent in the treatment of colon cancer and the same can certainly be confirmed through *in vivo* studies and clinical trials to confirm their effectiveness in patients.

Keywords: Colon cancer, molecular docking, stigmasterol, TACE, TNF-α.

Introduction

Colorectal cancer is one of the third commonly diagnosed cancers. This non-skin cancer is one of the major causes responsible for death in both men and women in developed countries. According to American Cancer Society, Cancer Facts & Figures (2014), there are 1,36,830 estimated new cases of colon cancer and 50,310 estimated deaths in both the sexes [**1**]. Colon is the part of the digestive system or gastrointestinal system. Most colorectal cancers originate from adenomatous polyp. Polyps are the growth that begins in the inner lining of the colon and moves toward the center. Most of the polyps are noncancerous. Only a few types of polyps can become cancer known as adenomas. Colon cancer grows slowly which takes 10-15 years [**2**]. Risk factors for this disease include physical inactivity, obesity, and high consumption of meat, smoking, and heavy alcohol intake. A family background of colon cancer or adenomatous polyps and personal history of inflammatory bowel disease are the other risk factors associated with colon cancer. Inflammation and tumorogenesis is a grievous combination, well supported by various researchers in last decade. Inflammation is a potential source of sporadic and genetic colon cancer.

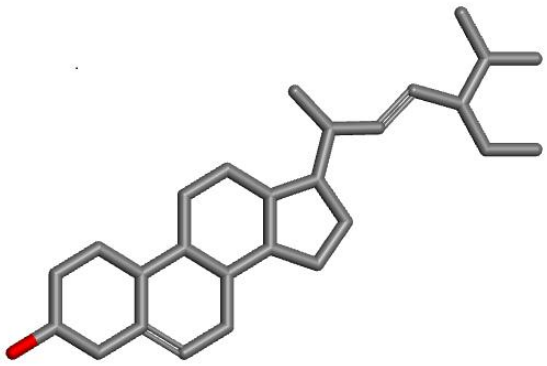

Fig. Structure of Stigmasterol.

Chronic inflammatory bowel disease ulcerative colitis is an important risk factor for the development of colon cancer [**3**]. Treatment of colon cancer with the oral drugs is possible only when diagnosed at the early stage of cancer development. Some FDA approved drugs for colon cancer are Avastin, Bevacizumab, Camptosar, Cetuximab, 5FU (Fluorouracil Injection). Surgery, radiotherapy, and chemotherapy are advised at the latter stage of colon cancer, which are associated with a reoccurrence of the disease and side effects like fatigue, constipation or diarrhea, a temporary or permanent colostomy, bladder irritation etc. Apart from the continuous development of new approaches to combat this baneful disease, not all the approved drugs can be prescribed for continuous long-term use due to drawing some adverse effects. Few advanced imaging techniques enabled the progression of treatment approach in diagnosis of hepatic metastatic disease and rectal cancer with the limitations like technical, economical and logistic challenges [**4**]. Besides the advancement of therapeutic strategies; treatment of colorectal cancer need to be more specific and effective [**5**]. On the contrary, medicinal plants or green material has a great potential to develop herbal drugs with very low or no adverse effects.

'Medicinal plants as Green material' is now becoming a major area of interest to the scientific society across the globe for drug development. Medicinal plants have the capacity to lower down the effects of cholesterol due to the presence of phytosterols. The most important phytosterols are sitosterol, campesterol, and stigmasterol. *Chlorophytum borivilianum*, 'Safed Musli', is one of the important medicinal plants having stigmasterol as major secondary metabolite with high medicinal value. *C. borivilianum*, also known as "the golden root", is an endangered herb that belongs to family 'Liliaceae'. Tuberous roots of this herb contain immunomodulatory and adaptogenic properties and are used to treat impotency, sterility and enhance male potency [**6**]. It is being used to cure physical illness and weakness, diabetes, arthritis, natal and postnatal problems, rheumatism and joint pains. This herb is an antimicrobial, anti-inflammatory and antitumor agent, also used to medicate diarrhea, dysentery, gonorrhea and leucorrhea. Medicinal properties and various biological activities viz. aphrodisiac, anti-oxidant, anti-cancer and immune booster are attributed by its major metabolite stigmasterol. Experimental studies suggest that due to having an anti-inflammatory effect, stigmasterol may offer protection from the most common cancers such as colon, breast, and prostate cancer. The chemopreventive effect of stigmasterol on *in vivo* cancer model was explored [**7**]. 5.34µg/mL stigmasterol was required to inhibit the activity of TNF-α [**8**]. TNF-α is a major immune-modulatory and proinflammatory cytokine that is synthesized as a membrane-anchored precursor. It supports the previous studies indicating the anti-inflammatory properties of stigmasterol, with low concentrations of tumor necrosis factor-alpha (TNF-α). Monocytes and macrophages are responsible for releasing TNF-α, involved in the host immune response [**9**]. Pro-inflammatory mediators such as TNF- α, IL-6, and nitric oxide are crucial for the uninterrupted functioning of immune system especially in the presence of any infection. On the contrary, it can be the reason for the development of tissue and organ injury when overproduced [**10**]. Increased concentration of TNF-α is responsible for the pathogenic process of infectious as well as autoimmune diseases [**11**]. TNF- α is the mediator of tumor-associated inflammation and tumorigenesis [**12**]. It was observed as an important mediator of initiation and advancement of colitis-associated colon carcinogenesis [**3**]. Colon inflammation was found to be responsible for the promotion colon TNF- α which is linked to increased chances of colon pathogenesis [**13**].

Various studies associate tumor necrosis factor alpha converting enzyme (TACE) with colon cancer [**9**]. TACE is the member of A disintegrin and a metalloproteinase-containing enzyme (ADME). It is responsible for producing the soluble form of TNF-α from its membrane-bound precursor i.e. pro TNF-α. An experimental study conducted on teleost proved TNFα-converting activity of TACE [**14**]. Various other studies proved the involvement of TACE in the suppression of colon cancer [**9**]. It is well known that high concentration of TNF-α is found in the cases of colon cancer. Inhibition of TNF-α production can suppress the signals that are responsible for releasing the mature TNF-α. Although a number of proteases have been shown to process pro-TNF-alpha, the most efficient are TNF-α converting enzyme (TACE). Since the enzyme plays an important role in converting TNF-α in soluble form, targeting the enzyme could be a potential therapeutic strategy counteracting the increase in TNF-α concentration, which has been seen in many cases of colon cancer. The present study explored a novel therapeutic perspective of stigmasterol for colon cancer in which overexpression of TNF-α was suppressed by stigmasterol through inhibition of TACE. Molecular docking studies have been used to identify the binding modes. A Molecular Dynamics (MD) simulation was carried out for the docked complex to analyze its stability inside the bodily conditions by using Gromacs simulation suite.

Experimental

Materials

Protein and ligand preparation

The crystal structure of TACE [PDB: 1BKC] was retrieved from Protein Data Bank. Structure of ligand molecule stigmasterol (CID: 5280794) was obtained from NCBI – PubChem Compound Database.

Prediction of active site

Understanding the reaction of catalytic residues is necessary in order to understand the accurate function of an enzyme. Although active site information of TACE was partially reported [**15**] earlier, current study presents more details on co-crystallized structure with its inhibitor and further validation has been done by *in silico* analysis. A most probable active site with amino acid residue information was obtained from Q-site Finder web server.

Molecular docking

Molecular docking was performed with AutoDock4 [16]. AutoDock4 is a one of the widely-used docking tool having an efficiency of predicting rapid and exact bound confirmations of ligand and their potential targets. AutoDock docking models are often consistent with X-ray crystal structures [**17, 18**].

MD simulation in water

A Molecular Dynamics (MD) simulation was carried out for the docked complex to analyze its stability inside the bodily conditions by using Gromacs simulation suite [**19**].

Methods

AutoDock uses a grid-based method for quick evaluation of binding energy of test confirmations. AutoDock starts with preprocessing of ligand by removal of coordinates from the PDB file and water molecules. AutoDock allows the target to be embedded in the grid while the investigating atom is consecutively directed to each grid point. Interaction energy of investigating atom and target molecule is calculated and is secured in the grid, which is then referred during docking simulation. Lamarckian genetic algorithm is used for conformational searching [**16**]. AutoDock4 uses a semi empirical free energy force field for the prediction of binding free energies of ligand to macromolecular targets. Q-site Finder anticipates the information active site residues by using energy criteria and computes van der Waals interactions of methyl probe with the target molecule. The clusters of favorable energies are graded according to the total interaction energies and the cluster of maximum energy filled the first rank [**20**]. Polar hydrogen was added to the protein structure to provide accurate ionization. The ligand was attributed to gasteiger charges and rigid roots. 13 Rotatable bonds were adjusted. Key residues were embedded in energy-container grid of 60 Å × 60 Å × 60 Å (x, y, z). The best confirmation was obtained through default Lamarckian genetic algorithm as a search protocol. The output from AutoDock4 was analyzed with Viewerlite 5.0. Ligplot was generated using PDBsum determined the number and length of H- bonds between the ligand and the receptor. The application of MD simulations to study the protein dynamics is well described in many previous studies [**21, 22, 23, 24**]. Here, we performed MD simulations for 30ns and studied the stability of protein as well as the ligand as a complex.

Result and Discussion

Identification of Active Catalytic site in TACE

Q-site Finder generated ten clusters based on probe and protein interactions after submitting the pre-processed structure of TACE. Individual probe site associate preferentially to the best-suited binding sites on the protein surface and these are the positions where a tentative ligand could interact and optimize its van der Waals interaction energy [20]. The obtained results included nearly 27 residues. All these residues are covered in the grid while performing docking. The active cleft of TACE has a catalytic zinc residue at its center pentacoordinated by the three-imidazole N_2 atoms of His 405, His 409, His 415. Along with these residues Glu 406, Met 345, Pro 437, Trp 312, Asp 344 around the zinc atom constitute the active site. The docked complex was forming a hydrogen bond with Gly 346 and showing hydrophobic interactions with many of the active site residues. **Fig. 1**(**a**) depicts the stigmasterol and TACE complex after molecular docking while the **Fig. 1**(**b**) is the ligplot showing hydrogen and hydrophobic bonds between ligand and macromolecule TACE.

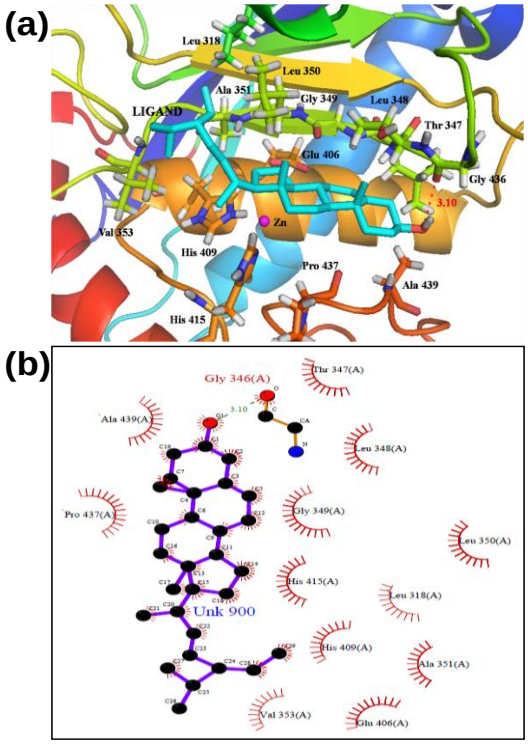

Fig. 1. (a) Stigmasterol and TACE Complex, (b) Ligplot showing hydrogen and hydrophobic bonds between ligand and macromolecule TACE.

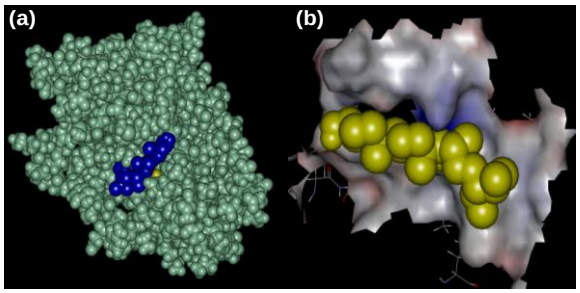

Fig. 2. complex1_tace rendered in cpk.

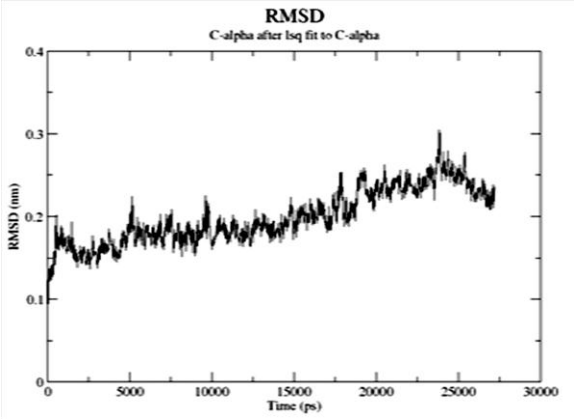

Fig. 3. Root mean square deviation.

Molecular docking simulation

We have investigated molecular docking and MD simulations on stigmasterol and TACE complex for the stability in the bodily conditions. CPK model is shown in **Fig. 2**. Computed Root mean square deviation (RSMD) (**Fig. 3**) suggests that docked complex is stable. Root mean square deviation was calculated by least square fitting of CA from the initial structure and found to be stable around 2A during simulations; suggesting that the docked complex is stable.

Ligand and the active site residues in the receptor domain within 8A distance were considered for the interaction studies. A schematic of detailed ligand atom interactions with the protein residues before MD simulations is shown here (**Fig. 4**). The docked complex was forming a hydrogen bond with Gly 346 and within the hydrophobic interaction range with many of the active site residues. Hydrogen bond distance between ligand and receptor is shown in **Fig. 5** (**a**). Hydrogen bonding pattern between ligand and receptor is indicating the formation of strong hydrogen bond during simulations. Hydrogen bond existence map for various amino acids interacting with ligand is given in **Fig. 5** (**b**). Cyan implies the presence of a hydrogen bond and Red implies the absence of one. The *y*-coordinate shows the hydrogen-bond index.

In the present study, we examined the molecular mechanism of the stigmasterol on TACE that helps in understanding of the cancer cell characteristics. We observed that stigmasterol docked onto active site of TACE. The active site of TACE comprises zinc atom coordinated by a conserved zinc-binding domain (405-HexGHxxGxxH-415) [**15**]. It is also determined by the three-dimensional structure of TACE through Q-site Finder server. The ligand is interacting with key amino acids Leu 348 and Gly 349 present in the binding pocket of the enzyme [**25**].

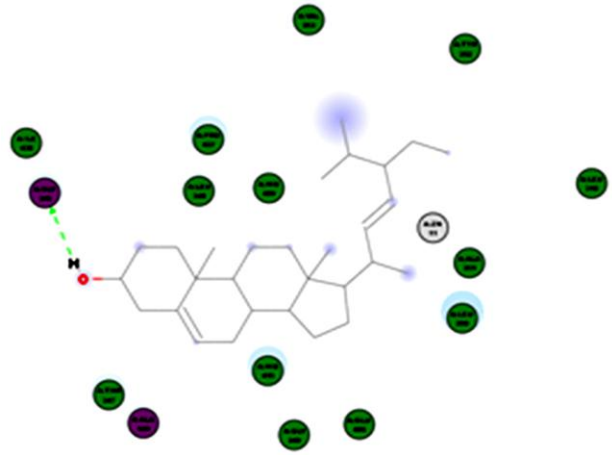

Fig. 4. A schematic representation of ligand interaction with the protein residues before performing MD simulations.

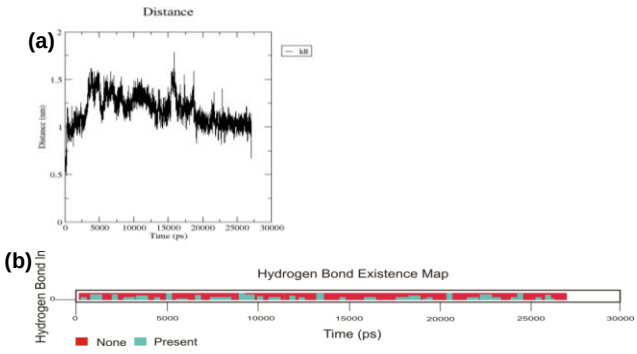

Fig. 5. (a) Hydrogen bond distance between ligand and receptor, (b) Hydrogen bond existence map.

The binding pocket also co-occurred with the binding site of stigmasterol acquainted in the co-crystallized structure acquired from PDB. Molecular docking score of the 3D structure of stigmasterol with the above-described active site of TACE was -10.04 predicting good binding affinity of TACE with stigmasterol. Docked complex exhibited molecular interaction with glutamate and histidine residues that play a key function during the proteolytic reaction procedure. Short hairpin RNA silencing TACE (shTACE) prevented and efficiently treated acute and chronic ulcerative colitis by decreasing TNF- α level [**26**]. The active site of TACE was blocked with highly selective new non-hydroxamate sulfonamide TACE inhibitors, considering as therapeutic targets for the treatment of TNF-dependent pathologies [**27**]. A quinazoline derivative therapeutically improved arthritis through suppressing production of TNF- α mediated by TACE [**28**]. Another study on TACE inhibition revealed TACE activation by non-receptor tyrosine kinase Src in

mechanically stressed cardiomyocytes. This process could be harmful to specific blockade of TNF- α secretion, which in turn leads to congestive heart failure [**29**]. Considering above facts, the interaction of TACE and stigmasterol were anticipated to intervene with the interaction of substrate for the binding site of TACE, hence strengthening the idea of stigmasterol as TACE inhibitor.

Molecular dynamics simulation is a reliable and new way for the prevention of many diseases [**30**]. Molecular dynamics simulations are a widely used computer simulations technique to study the conformational dynamics of biological macromolecules such as proteins [**31**]. Molecular dynamics of the docked complex were examined to find out its stableness in bodily conditions. A simulation run time was 30ns, which was sufficient for the rearrangement of a ligand-bound protein molecule to acquire a stable binding manner. Root mean square deviation was calculated by least square fitting of CA from the initial structure and found to be stable around 2A during simulations; suggesting that the docked complex is stable. A structure showing the stationary phase was considered to study the molecular interaction pattern in the docked complex. H-bond, hydrophobic interaction, and van der Waals interaction were the factors that play an important role to make the stigmasterol and TACE a stable interaction. The dynamic stableness of stigmasterol during simulation runtime and its interaction with the important residues present at the binding site of the enzyme possibly consolidate the mode of action of stigmasterol on inhibition of TACE, which is responsible for the release of the soluble form of TNF-α.

Drug-likeliness

The successful evaluation has been done for drug-likeness based on Lipinski's "Rule of Five", while 46 computed physicochemical properties or molecular descriptors were used to predict ADMET (absorption, distribution, metabolism, elimination and toxicity) of the compound.

Conclusion

Molecular docking and simulation study revealed that presence of a ligand in the active site of TACE, interacting with the key residues consolidated the idea that stigmasterol is a potential inhibitor of TACE. Furthermore, this study is a stepping-stone in order understands the prevention mechanism of TNF-α, a high concentration of which is responsible for colon cancer. Hence, it can be used for further studies as a natural anti-cancer drug.

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Author's contributions

Conceived the plan: N.J., N.B., N.S.; Performed the experiments: N. J., N.S.; Data analysis: N.J., G.M., N.S., N.B; Wrote the paper: N.J., N.S., N.B. Authors have no competing financial interests.

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