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Short Communication

Identification of Gingerol (6-Gingerol) as Humming Inhibitor of Cancer through Docking Analysis

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Abstract

Cancer is a hyper-proliferative disorder, which induces deformation of a natural cell by genetic mutations in DNA. Due to the high death rate associated with cancer, it is a major health problem for developing countries. Chemotherapy, Radiotherapy, and Chemically derived drugs are used in the treatment of tumors, however, researchers are finding out alternate methods for chemotherapy and radiation therapy. Ginger is a medicinal plant and is used as an anti-viral, anti-fungal, anti-parasitic, antioxidant, and antibacterial agent. It possesses anticancer properties also. Most of the ginger ligands showed good interaction with the selected target but based on *in silico* analysis ADME (Absorption, Distribution, Metabolism, and Distribution) 6- gingerol was found superior due to its solubility and neurotoxic effect in comparison to the other compounds. The cytotoxicity of ginger increased with the increment of the concentration and its lower doses have preventive properties. The IC_{50} value recorded for both cancer cell lines after 22h treatment (Human Breast Cancer, colon Cancer) showed uniform cytotoxicity in both the cell lines studied. The study highlights the potential of 6-gingerol for drug development against cancer

INTRODUCTION

Cancer is a major health problem resulting in 10 million deaths in 2020 (WHO, 2022). Most common cancers are breast, lung, colon, rectum and prostrate and many of them can be treated effectively if diagnosed and detected early [1,2]. Nuclear factor –kappa β (NF-k β) and activator protein (AP-1), Epidermal Growth Factor Receptor (EGFR), cMet, Phosphoinositide 3-kinase biomarkers express through different types of cancers. C-Met has been shown to be deregulated and associated with high tumor grade and poor prognosis in a number of human cancers [1,2]. Deregulation of the receptor tyrosine kinase c-Met has been implicated in several human cancers and is considered as an attractive target for small molecule drug discovery [3]. It has been found that the enzyme family (P13k) is actively involved in cellular functions like proliferation, deregulation, cell growth, motility and that are indicator of cancer [4]. Various studies have revealed that PI3k inhibitor can be of great importance in finding anticancer drug in future [4]. NF-kβ transcription factors play an important role in the inducible regulation of genes involved in the proliferative responses of cells [5,6]. Cyclooxygenases -2 is a form of non-steroidal anti-inflammatory compound which is commonly known as COX-2 enzymes and is an inducible prostaglandin. It has been found out promising target for the treatment of various human cancers [5]. Dis-regulation of NF-κβ is playing a major role to cancer inflammation and autoimmune diseases, viral infections, and irregular immune development. AP-1 activation is related to growth regulation, cell transformation, and immature immune response and has been found in regulation of genes involved in apoptosis and proliferation [6]. Targeting protein AP-1 or its activating enzyme could be effective agents for the treatment of several cancers [7]. Due to the high rate of death caused through different types of cancers and the serious side effects of its treatment (chemotherapy, radiation), the scientist is finding alternative and complementary methods of treatment [7,8]. Herbal medicines have long been used and are still being used especially in developing countries by tribal communities, Ayurveda practitioners as the major source of tumor and other disease treatment, mainly because of their little or no side effects in comparison to chemotherapy and other treatments of cancer [8]. Herbaceous medicinal plants have been used in the form of medicine for their natural antiseptic properties [8], however, they need scientific validation and the world over the researchers are working on validation of ancient knowledge and practices by scientific means [9].

Ginger is one of the most valuable medicinal plants and is known for its spicy and medicinal properties [10]. It has been used as an anti-viral, anti-fungal, anti-parasitic, antioxidant and antibacterial agent with potential anti-cancerous activity as shown in (Figure 1) [11,12].

The value of ginger has been increased due to its pharmacological application and its nature of low toxicity [12,13]. Ginger rhizome contains 400 identified volatile oil and nonvolatile compound [14]. Several natural compounds like sesquiterpenes-9 e.g. zingiberene, β-sesquiphellandrene, curcumene), fernesen and monoterpenes are found in ginger but gingerol is one of the most important pharmacologically active compound that possess several medicinal activities like analgesic, antipyretic, gastro protective, cardio tonic and anti-hepatotoxic *etc,* [15]. 6-gingerol is considered potent and pharmacologically active compound and now used as a target for drug development also. Gingerols contain a β-hydroxy keto group in its structure which makes them thermally labile and undergo dehydration readily to form the corresponding shogaols [15,16]. Pharmacological investigations have revealed that ginger and its major ingredient have chemo-preventive and chemotherapeutic effects on several types of cancer cell lines and on animal models [17]. The present study focuses to identify cancer targets for gingerols and shogoal using *in silico* tools and to validate anti cancerous properties of gingerol.

MATERIALS AND METHODS

This papers *in silico* analysis of potential cancer target for gingerols using docking program to identify the bonding between ligands and tumor target, followed by comparison of ligands with pre available cancer drugs to validate the ant-cancerous properties of gingerol with different types of tumor cell lines [18].

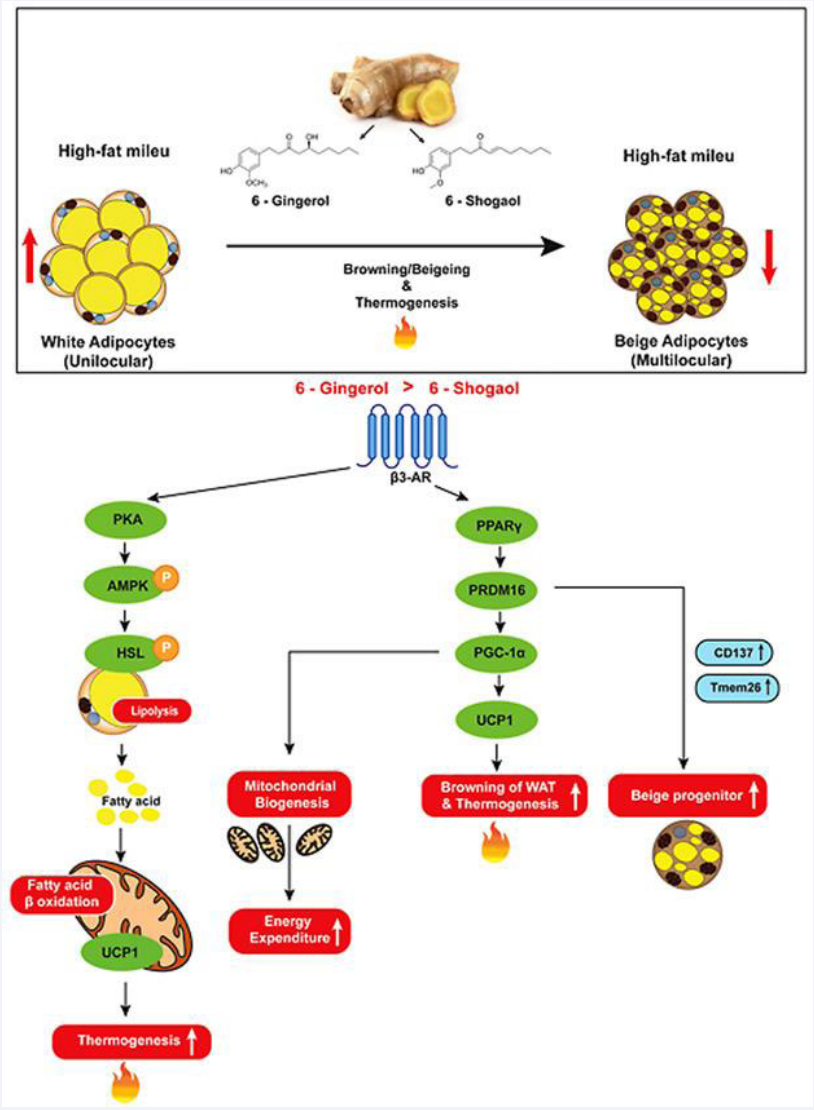

Figure 1 Structure of 6-Gingerol and its effects on living cells [31]

Then, we retrieved the 3D structure of ginger ligands, 6-gingerol, 8-gingerol, 10-gingerol and shogaol and the two validated anti-cancer drugs-Quercitrin and Antabuse whose structure is already available on PubChem it's an online database [19,20].

Preparation of ginger ligands and drugs and their filtrations

Structure of ginger ligands and drugs was prepared using Marvin Sketch Software which is freely available, and is helpful in the removal of duplicates, enumerated tautomer's/isomers, added hydrogen bonds and minimized energy using CHARMM tool. For the identification of drugs like properties of ligands, we filtered the structure of ligands with approved drugs [21]. Filtration of prepared ligands and approved drugs were made using Pfizer's rule that sets the criteria for drug like properties (molecular weight <5 Daltons, number of hydrogen bond donors <5, number of hydrogen bond acceptors <5, and partition coefficient (LogP) <5). After passing the Pfizer's rule, the molecular weight of a chemical compound was calculated. After filtration of ginger ligands and approved drugs, molecular docking was performed with selected cancer targets [17].

Preparation of target protein molecules and prediction

Preparation of the retrieved protein was performed using viewer lite 5.0 tools & chimera which helps to design the protein structure by removing extra chains of target protein and the removal of crystallographic water molecules [22]. Thereafter, hydrogen atoms were added to make perfect chemistry of protein structure and to minimize the energy of protein structure for stable conformation using CHARMM. This minimized structure was used as a template for docking program. The active site and binding mode of ligands in the selected region were analyzed using the Dock Blaster [23,24].

Molecular docking

Docking program was performed using Dock Blaster between target proteins of cancer with four ginger ligands. NF-k beta and AP-1 were also docked with approved drugs-Antabuse and Quercitrin (19). It contained minimum difference between interaction energy and was considered as the best interaction between ligand and target, along with lowest binding energy calculated as the scoring function [25]. Number of hydrogen bonds between the targets and the ligands were also calculated by using R-Square tool. The optimal distance between two atoms connected by a hydrogen bond was set to 1.5 Å with a tolerance of 0.3 Å [21].

Toxicity evaluation

DOCK Blaster was used to find out the pharmacokinetic, pharmacology parameters and to assess the quality of the molecules in the form of absorption, distribution, metabolism, excretion and toxicity after human ingestion. This method reduces the cost and chances of clinical failures of new drugs. The parameters calculated by this descriptor included aqueous solubility, Human Intestinal Absorption, t5cytochrome P450 inhibition, Blood-Brain-Barrier (BBB) penetration and toxicity levels with the help of R-Square *inslico* analysis tool.

Maintenance of cell lines and cytotoxicity assay

On the basis of docking score, the toxicity analysis of 6-gingerol was carried out for *in vitro* cytotoxicity assay using two different cancer cell lines which included, Human Colon Cancer (HCT15), Human Breast Cancer Cells (MCF-7) which were obtained from DRDO [22]. 6-gingerol standard (HPLC grade, 95% pure) was procured from Medlock healthcare. The cells were cultured in RPMI-1680 medium supplemented with 12 % fetal bovine serum (FBS), sodium pyruvate and antibiotic (penicillin and streptomycin) at 35°C. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 4.5 g glucose, 1% each HEPES buffer, 5-diphenyltetrazolium bromide) assay was done to evaluate the proliferative capacity of cells. A 60 well plate was used with 100 µL medium containing cancers cells. After 45 h of incubation, the cells were treated with gradient concentration (17, 34, 68, 102, 136 and 170 µM) of 6-gingerol which was dissolved in ethanol. The experiment was repeated twice for human breast cancer and human colon cancer cells. Observations were recorded at 24 h intervals. The spent medium was removed and 100 µL of fresh medium with 10 µL of MTT (5 mg/ml in PBS) were added to the wells and cells were incubated at 35°C in dark for 6 h. The Formosan product was dissolved by adding 100 µL of DMSO. The absorbance was measured at 570 nm using monochromatic ELISA.

RESULTS AND DISCUSSION

Retrieval of ginger ligands, approved drugs and identified cancer targets

3D structure of four ginger ligands *viz.* 6-gingerol (ZINC42793), 8- gingerol (ZINC68114), 10-gingerol (ZINC68115), 6-shogaol (ZINC81794) and Antabuse (ZINC58459) and Quercetin (ZINC1178) downloaded from Zinc database. Expect Antabuse all ligand have a benzene ring. 3D structures of targets cancer protein (6EOQ) were retrieved from PDB (Protein Data Base) database [23]. The structure of target protein was retrieved on the basis of X-ray diffraction method and their resolution capacity [24].

Preparation of Cancer Target protein, approved drugs and ginger ligands cancer

Cancer ligands were prepared using *in silco* tools Marvin Sketch to change the ionization, and 3D coordinates. After preparation of ligands these ligands were filtered based on Pfizer's rule, [Table 1]. 6- gingerol, 6-shogaol passed this rule while other failed. On comparison it was found that Antabuse passed this rule while quercetin failed.

All non-standard residues were removed from the target proteins during protein modeling. Then we performed the processor of energy minimization which helped to reduce the

Table 1: Filtration of ginger ligands and drugs using Pfizer's rule

Compound name	Partition coefficie nt (XLogP3)	Hydrogen Bond Donar (No.)	Hydrogen Bond Acceptor (No.)	No. of rotation bonds (No.)	Pfizer's rule
6- Gingerol	< 4.5	< 4.7	< 10	11	Pass
8- Gingerol	< 4.5	< 4.7	< 10	12	Fail
8- Shagaol	> 6	< 4.7	< 10	13	Fail
10- Gingerol	< 4.99	< 4.7	< 10	10	Pass
Quercetin	< 4.7	< 4.7	< 10	8	Pass
Antabuse	< 4.7	>5	> 10	3	fail

effect of potential energy, vander-waals energy and electrostatic energy. The maximum numbers of active sites from prepared proteins were found, nine in 3LN1 (COX-2) and two in 4HMY (AP-1) in this protein model. Only one active site was selected from each target for docking analysis which had maximum number of similar amino acid residues in their active site.

Molecular docking analysis

Four ginger ligands and two approved drugs (Antabuse and Quercetin) were docked with identified cancer targets using DOCK Blaster as shown in (Figure 2). The minimum difference between interaction energy was observed in case of ginger ligands and targets as 2.256 Kcal/mol (6-gingerol with c-Met), 0.8043 kcal/mol (8-gingerol with c-Met), 4.9515 kcal/mol (10-gingerol with PI3k), 6.6666 kcal/mol (6-shohaol with PI3k). In case of approved drugs the difference was around ~5 kcal/mol. The binding energy of identified targets with 6-gingerol ranged from -62.2134 to -157.2092, 8-gingerol from -21.9807 to -140.5949, 10-gingerol from -87.531 to -131.1699 and 6-shogaol from -38.7325 to -107.683. Among the ginger ligands, 6-gingerol showed the lowest scores of -107.8914 Kcal/mol, -65.6825 kcal/mol and -76.0004 kcal/mol while interacting with EGFR, c-Met and NF-kβ respectively at the residues LYS745, MET789, MET1160, PRO1158 and ASn20 of active site and Receptor cavity with hydrogen bond lengths 1.8 Å, 2.0 Å, 1.9 Å, 2.3 Å and 2.2 Å respectively. In case of PI3k and COX-2 lowest score (-87.5317 and -89.9435) was observed while interacting with ginger ligand 10-gingerol at the residues SER806, LYS833, LYS890, ASP964, HIS200 and ASN368 with hydrogen bond lengths 2.2 Å, 1.8 Å, 2.4 Å, 2.2 Å, 2.0 Å, 1.9 Å and 2.1 Å respectively. In case of AP-1, 8-gingerol showed lowest score (-140.5949) while interacting at the residues LYS127, THR45 and ILE46 with hydrogen bond lengths 1.8 Å, 2.8 Å and 2.2 Å respectively.

EGFR was seen over expressed in a variety of cancer like NSCLC [22], prostate cancer [23]. c-Met is found over expressed in variety of cancers like, breast cancer [24] and human colon cancer [25]. NF-kβ activated in different types of solid tumors like prostate, breast, cervical, pancreatic, gastric, ovarian and lung cancer [26,27]. PI3k is a signaling molecule that plays a critical role in regulating apoptosis. Mutated phosphoinositide 3-kinase causes cancer development, is highly activated in variety of cancer like, gastric, colon, breast, pancreatic, prostate, cervical, ovarian, skin and lung cancer [27,28]. COX-2 is over expressed in every premalignant and malignant condition colon, liver, pancreas, breast, lung, bladder, skin, stomach, head and neck and esophagus [29,30].

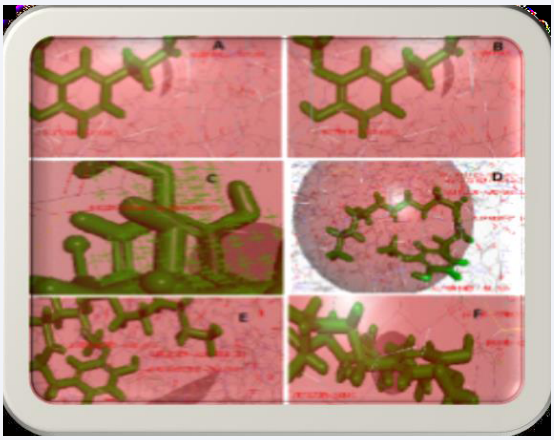

Figure 2 Docking analysis of 6- gingerol with cancer target.

Drug analysis

The ADME analysis of ginger ligands and approved drugs are listed in [Table 2]. Adsorption, Distribution, Metabolism, Excretion and Toxicity (ADME) descriptor levels of the analogs were obtained from the ADME Descriptors protocol of DS 4.0 which is presented in [Table 2]. Among the ginger ligands 6-gingerol showed good solubility and adsorption with medium BBB level, nontoxic and non-inhibitor of the enzyme CYP2D6 in metabolism of xenobiotic in the body. In case of approved drugs, Antabuse showed low solubility, good absorption, very high penetration, but toxic and non-inhibitor while quercetin showed good solubility, but very poor absorption, very low penetration, toxic and non-inhibitor of the enzyme CYP2D6 in metabolism of xenobiotic in the body. Based on docking result and ADME/toxicity analysis, 6-gingerol was found superior among ginger ligands and approved drugs. Hence 6-gingerol was carried forward to validate the anti-cancerous properties through cell culture studies.

In vitro cytotoxicity of 6-gingerol

MTT assay was performed to determine the cytotoxicity of 6- gingerol on HCT15, L929 and Raw 264.7 cells with 17, 34, 68, 102, 136 and 170 M concentrations. 6-gingerol was found to inhibit the cell growth in all test cells lines. The viability of the cells decreased significantly with 6-gingerol in a dose dependent

Table 2: ADME/Toxicity properties of ginger ligands and approved drug.

Compounds name	ADMET Solubility level	ADMET Absorption level	ADMET BBB Level	Hepatotoxic prediction	CYP2D6 Predictio
6- gingerol	3	0	2	False	False
10-gingerol	3	0	1	False	False
8- gingerol	3	0	1	False	False
6-shogaol	2	0	1	False	False
Antabuse	2	0	0	True	False
Quercitrin	3	3	4	True	False

manner. Cytotoxicity of 6-gingerol on different cancer cell lines at different concentrations after 20 h.. The IC50 value of 6-gingerol on different cancer cell lines viz. HCT15, L929 and Raw 264.7 was observed at 100 µM, 102 µM and 102 µM respectively 24 h. After treatment, we observed the reduction in cell viability at different concentration of 6 Gingerol as shown in (Figure 3) and [Table 3].

Sixteen percent reduction was observed in cell viability at 10 µM concentrations of 6-gingerol and 6-paradol [29] when anti-cancerous effect was studied in MDA-MB-231 cells (breast cancer). In present investigations, 13% reduction in cell viability was observed in L929 (murine fibro sarcoma cell), 25% in HCT15 (colon cancer cell) and 26% in Human Breast Cancer at 17 µM concentration of 6-gingerol.

CONCLUSIONS

Cancer is the leading cause of death worldwide and majority of deaths are due to lung, stomach, liver, colorectal and female breast. The present investigations found the effectiveness of 6-gingerol as an anti-cancerous phytochemical compounds through molecular docking and cell culture studies and to highlight the potential of 6-gingerol for drug development against cancer. Pharmacological investigations have revealed that ginger and its major biomolecules have chemo-preventive and chemotherapeutic effects on a variety of cancer cell lines. As 6-gingerol is identified as a very good phyto-compound compared to other ginger ligands like 8-gingerol, 10-gingerol and 6-shogaol and approved drugs like, Disulfiram and Quercetin, research thrust may be focused on drug development using 6-gingerol against cancer.

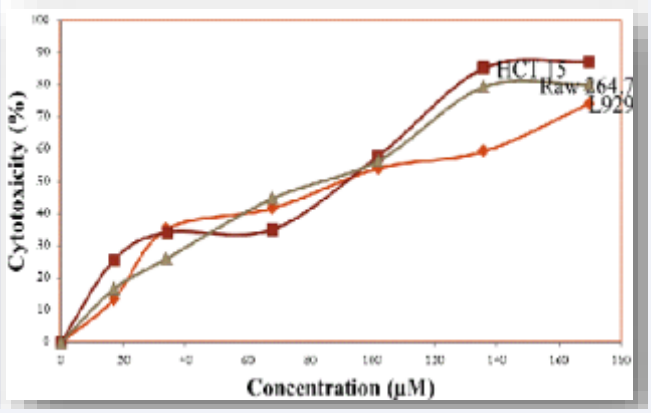

Figure 3 Cytotoxicity of 6-gingerol in different cell lines after 24 hours treatment.

Table 3: Effects of 6-ginerol on cytotoxicity of cancer cell lines after 24 hour treatment. Percentage of dead cells calculated over control. (Percentage of dead cells in control=0 Control is the cell line without 6- ginerol and percentage of dead cells observed in control is zero)

Concentration of 6-gingerol (µM)	Percentage of dead cells		
	L929 (Murine fibro sarcoma cell),)	HCT15 (Human colon cancer)	Human Breast Cancer
17	13.33	25.40	26.33
34	34.16	34.13	48.09
68	41.61	33.03	64.64
100	54.00	51.87	60.33
106	60.32	86.17	78.64
172	72.22	87.00	85.15

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