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Toxicological analysis on EGFR Protein Inhibitors (Clerodane) using TOPKAT analysis.

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ABSTRACT

The epidermal growth factor receptor (EGFR) is under investigation as a therapeutic target for cancers. Lung cancer cell lines are variably dependent on autocrine stimulation of EGFR since it has a role in signal transduction .We therefore examined the effects of a selective EGFR tyrosine kinase inhibitor Clerodane. Clerodane molecule was used as an inhibitor for EGFR and it was extracted from *Tinospora cordifoli(common name-Guduchi)*. These compounds after docking with EGFR protein were found to possess good energy score and also highly inhibited the protein molecule showed anticancer activity on EGFR. Clerodane highly inhibited the protein EGFR at the position of ARG 231 & CYS 224 . ADME properties of Clerodane by using ADME tool of TOPKAT (DS 2.5) and the Pharmacokinetics results for Clerodone molecule indicated that molecule is non toxic effect to female mouse and female rat (Norms per NTP carcinogenicity). Moreover, as per Food and Drug Administration carcinogenicity value, there is no toxic effect on male rat and male mouse. The pharmacokinetic results showed normal absorption rate, solubility, heptotoxicity, CYP2D6 and PPB values.

KEYWORDS: EGFR, TOPKAT, PPB, BBB, Clerodane

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INDRODUCTION

The activities of epidermal growth factor receptor (EGFR) have been identified as key drivers in the process of cell proliferation, metastasis, angiogenesis, and dedifferentiation [1]. It has been shown that the EGFR-mediated drive is increased in a wide variety of solid tumors including lung cancer, prostate cancer, breast cancer, gastric cancer, and tumors of the head and neck. Heightened activity at the EGFR is caused by an increase in the concentration of ligand around the cell, and an increase in receptor numbers or receptor mutation can lead to an increase in the drive for the cell to replicate. It has been postulated that agents designed to block EGFR will inhibit signal transduction, resulting in multiple antitumor mechanisms[2], as well as enhancing chemotherapy and radiotherapy antitumor effects.Lung Cancer Causes and Treatment: - Lung cancer is usually caused by smoking or exposure to second-hand smoke**.** Researcher estimates that more than 90% of lung cancer in men and at least 70% in women are caused by cigarette smoking. Normally, there is a thin layer of mucus and thousands of tiny hairs(cilia) lining the inside of our breathing tubes within our lungs. The mucus and cilia act as a natural cleaning system for our lungs.If we breath in dirty or polluted air, the little bits of dirt, the cilia move together like a wave to push the dirt-filled mucus out of your lungs.Then we cough, spit up, or swallow the mucus, and the dirt is out or your lungs(Ref-1).Smoke from cigarettes, cigars and pipes is made up of over 4,000 dangerous chemicals; many of these chemicals are carcinogens .smoking damages our lungs by delivering these chemicals inside our body , and causes damage to lungs and effects its natural cleaning and repair systems. Thus dirt and pollution get stuck into lungs, and stay there. As dirt and smoke chemicals bulid up in lungs, increases the higer change of getting lung cancer. The more you smoke, or exposed to cigarette smoke,the greater your risk of getting lung cancer. Depending upon what kind of lung cancer you had, and what stage cancer is it can be treated with :-

i) ***Surgery*:-** Pneumonetomy, Lobectomy, Segmenectomy, Laser surgery
ii) ***Radiation therapy*:-**It uses high energy radiation to kill cancer cells.
iii) ***Chemotherapy*:-** It means taking anti-cancer drugs.
iv) ***Targeted therapy*:-**This is a word for drugs that attack pathways specifics for cancer cells.

3. Role of Epidermal Growth Factor Receptor in Lung Cancer[9]*:-*

The EGFR is a transmembrane receptor with an extracellular ligand-binding domain, a helical transmembrane domain, and an intracellular tyrosine kinase domain[7]. Activation of EGFR by epidermal growth factor (EGF) and other ligands (amphiregulin, TGF-α) which bind to its extracellular domain is the first step in a series of complex signaling pathways which take the message to proliferate from the cell membrane to the genetic material deep within the cell nucleus [3].

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Activation of EGFR by epidermal growth factor (EGF) and other ligands (amphiregulin, TGF-α) which bind to its extracellular domain is the first step in a series of complex signaling pathways which take the message to proliferate from the cell membrane to the genetic material deep within the cell nucleus[4]. EGF is a small mitogenic protein that is thought to be involved in mechanisms such as normal cell growth, oncogenesis, and wound healing[5]..

A clerodane has effective compound for anticancer activity[9] and diterpene (or clerodane diterpenoid) is a compound terpene derivative, more precisely a bicyclic diterpenoid. It is rather a rare form of diterpenoid and is structurally related to the bicyclic diterpenoids labdane and ginkgolide. It is synthesized in the

chloroplasts by plants mostly belonging to families Lamiaceae and Asteraceae. Gefitinib trade name Iressa (Grade 1 and 2), marketed by AstraZeneca and Teva, and is a drug used for the treatment of breast, lung and other cancers. Gefitinib is an EGFR inhibitor, like erlotinib, which interrupts signaling through EGFR in target cells. Therefore, it is only effective in cancers with mutated and overactive EGFR.

In the present study we examined the toxicological effects of a selective EGFR tyrosine kinase inhibitor Clerodane and Iressa 2 on normal mice and rats.

MATERIALS AND METHODS

Receptor:- Epidermal Growth Factor Receptor Protein

PDB ID: - 1 MOX

Crystal Structure of Human Epidermal Growth Factor Receptor (Residues 1-501) in complex with Tgf-Alpha [Transferase GROWTH FACTOR, EC: 2.7.1.112]

- Taxonomy: Homosapiens
- Proteins: 4
- Chemicals: 8
  modified: 2007/11/02; MMDB ID: 24621
- Active Site:
  GLU2,GLU3,LYS4,VAL6,VAL36,GLU60, GLN81,ARG84,GLU221,SER222,ASP2 23,CYS224,LEU225,VAL226,CYS227,L YS229,PHE230,ARG231,GLU232,GLU 233,ALA234,THR235,CYS236,ALA265, THR266

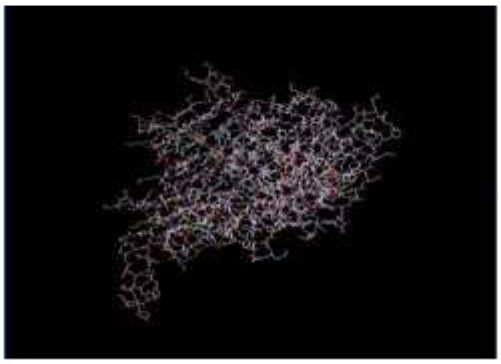

Figure 1
Protein molecule

Clerodane

Clerodane molecule was used as an inhibitor for EGFR and it was extracted from *Tinospora cordifolia*. **(**5R, 10R)- 4R, 8R- dihydroxy-2S, 3R:15,16- diepoxycleroda- 13(16),17,12S,18,1S- dilactone)

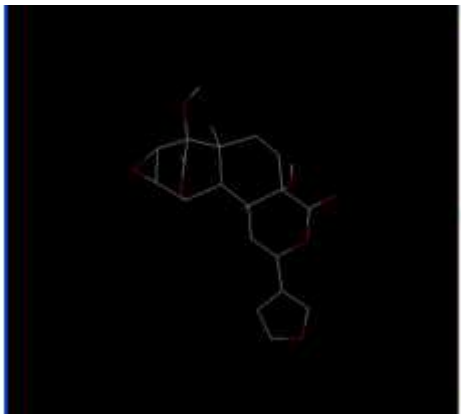

Figure 2
Clerodane (ligand)

TOPKAT analysis

TOPKAT analysis calculates the predicted absorption, distribution, metabolism, excretion and toxicity (ADMET) properties for collections of molecules such as synthesis candidates, vendor libraries, and screening collections. The calculated results were used to eliminate compounds with unfavorable ADMET characteristics and evaluate the proposed structural refinements, designed to improve ADMET properties like aqueous solubility, blood brain barrier penetration, plasma protein binding, CYP2D6 binding, Hepatotoxicity and Filter sets of small molecules for undesirable function groups based on published SMARTS rules prior to synthesis. Results are expressed in relation to NTP carcinogenicity and FDA carcinogenicity by pharmacodynamic and pharmacokinetic methods.

RESULTS AND DISCUSSION

The toxicological analysis of clerodane and Iressa using TOPKAT (DS 2.5) are summarized in Tables 1, 2 and 3.

Table 1
NTP Carcinogenicity FDA carcinogenicity

Comp	Male Rat	Female rat	Male mouse	Female mouse
Clerodane	0.990	0.447	1.000	0.000

Table 2
Pharmacokinetic studies using ADMET (DS 2.5)

Comp	BBB LEVEL	ABSORPTION LEVEL	SOLUB LEVEL	HEPATATOXICITY
Clerodane	3	0	2	0

The pharmacokinetics results have shown that clerodane molecule is non toxic to female mouse and female rat (Norms per NTP carcinogenicity). It was also non toxic to male rat and male mouse according to Food and Drug Administration carcinogenicity standards. The results have clearly indicated that clerodane is less toxic to the lab animals used in the present study. The absorption rate, solubility, heptotoxicity, CYP2D6 and PPB values were also found normal (Table 4).

Table 3
Blood Brain Barrier

Comp	BBB LEVEL	ABSORPTION LEVEL	SOLUB LEVEL	HEPATATOXICITY
Clerodane	U	G	G	NT
Iressa 2	L	G	G	NT

Note: U-Undefined G-Good NT- Not toxic

Table 4
Summary of pharmacokinetic studies using ADMET (DS 2.5)

PK characters	Ranges
Absorption rate	good
Solubility	good
Heptotoxicity	Non toxic
CYP2D6	Non inhibitor
PPB	No markers flagged and AlogP98 < 4.0

CONCLUSION

The current study has clearly shown that the compound Clerodane has good pharmacokinetics and pharmacodynamic results, and it can be used as a drug similar to other molecule. However, more research has to be done to confirm its efficiency.

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