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Study of Binding Modes of Active Ingredient of Licorice on TGF-β receptor: A Molecular Docking Approach

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Abstract

Background: Oral submucous fibrosis (OSMF) is a collagen disorder primarily mediated by Transforming Growth Factor-β (TGF-β). But still there exists no definite cure for this disease. Phytochemicals have become increasingly popular as treatment approaches in OSMF owing to the lack of adverse effects. Licorice is one such phytochemical that is thought to possess anti-fibrotic potential in-vitro in liver and lung fibrosis models. But the exact role of licorice in blocking the fibrotic cascade is yet to be elucidated. Molecular docking is an in silico procedure that is used to assess the interaction between the two molecules and thus could aid in the discovery of agents that could have potential pharmacological effects.

Aim: To virtually evaluate the binding efficacy of active compound present in licorice on TGF-β receptor type 2 by using a molecular docking approach to assess its antifibrotic potential.

Materials and methods: Three active compounds of licorice were identified to have antifibrotic potential based on PUBMED, EMBASE and Cochrane library databases. The chemical structure of the active compounds (ligands), Glabridin, Glycyrrhizin and glabrene and the target molecule, TGF-β receptor 2 were then retrieved from PubChem and Protein Data Bank (PDB) databases respectively. Molecular docking of the ligand and target molecule was then performed using SYBYL2.0® software.

Results: Glabrene, an active compound present in licorice showed the greatest binding affinity to TGF-β receptor type 2 molecule when compared to the other ligand molecules. A single hydrogen bond was formed for Glycyrrhizin and glabridin while four hydrogen bond were formed between glabrene and the TGF-β type 2 receptor molecule.

Conclusion: Our study showed that Glabrene has high binding affinity with TGF-β suggesting the possible role

of Glabrene in inhibiting TGF-β mediated fibrosis. Further in-vitro studies are however required to assess if they inhibit TGF-β and subsequent fibrosis in arecoline induced fibrosis models.

Keywords: Oral submucous fibrosis, Licorice, Glabrene, TGF-β

Introduction

Fibrosis is the process of excess accumulation of extra cellular matrix within organs and tissues and is involved in the pathogenesis of various chronic diseases. Oral submucous fibrosis (OSMF) is one such disease and is characterized initially by inflammation which when left unchecked results in gradual hyalinization of the lamina propria and deeper tissues of the oral cavity. Due to progressive fibrosis, stiffness ensues which ultimately results in an inability to open the mouth thus restricting food intake and impairing speech[1]. Transforming growth factor- β (TGF-β), a key mediator of fibrosis is up-regulated in OSMF. Fibrosis in OSMF is initiated through the TGF-β/Smad signaling pathway which in turn activates a cascade of events that cause hyalinization of the tissues[2]. Though numerous treatment modalities have been tried time and time again, there is no definite cure for this disease and if left untreated this disease could also undergo malignant transformation.

Phytochemicals have become increasingly popular as treatment approaches in OSMF owing to the lack of adverse effects. Licorice is one such phytochemical with antifibrotic potential. Licorice, derived from the root of *Glycyrrhiza glabra*, is native to the Middle East, southern Europe, and India. It is a commonly used sweetener in the food industry. In India, licorice has long since been used in Ayurveda for the treatment of minor ailments. Numerous studies have been done to evaluate the anti-fibrotic property of licorice in hepatic and renal fibrosis models. Evidence suggests that Glycyrrhizin, an active compound present in licorice directly interferes with TGF-β, the key mediator of fibrosis[3], [4]. Flavonoids like glabridin also have antifibrotic potential. Though studies have been performed to study the efficacy of these phytochemicals in various models of fibrosis, the exact mechanism of action of these active compounds is yet to be illuminated on the molecular level.

Molecular docking is a subset of bioinformatics which is used to study the relationship and interaction between two or more molecules at the molecular level. It is used to predict the three-dimensional configuration of any complex formed when a ligand binds to a specific target molecule. Molecular docking not only helps to characterize the behavior of the ligand molecules but also helps in the identification of the binding sites on a target molecule which would inturn help in the elucidation of fundamental biochemical processes involved.

In the current era, attention has shifted towards alternative medicine in the treatment of various disorders including OSMF. But prior to performing wet bench studies, molecular docking could aid in the screening and identification of appropriate ligands possessing anti-fibrotic properties. Till date the paucity of literature available to assess the antifibrotic potential of licorice at the molecular level still limited. Thus, the current study aims to virtually evaluate the binding efficacy of active compounds present in licorice on TGF-β receptor type 2 by using a molecular docking approach to assess its antifibrotic potential.

Materials and Methods

Selection and preparation of ligand and target: An extensive literature search was done using PUBMED, EMBASE and Cochrane library databases to identify the active compounds of licorice possessing antifibrotic potential using key words such as “Licorice”, “TGF-β”, and “Fibrosis”. Three active compounds of licorice-

Glabridin, Glycyrrhizin and glabrene was identified and were chosen to be used as ligand molecules.

The chemical structure of the chosen ligand molecules were then retrieved from Pubchem database in SDF file format. The target molecule chosen was TGF-β receptor type 2. The 3D structure of the target molecule was downloaded from the PDB database in SDF file format.

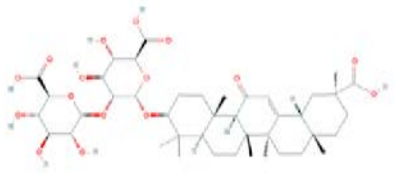
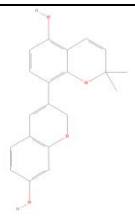
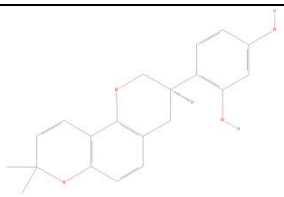
The files downloaded in SDF format were then converted to sybl mol2 file formats using Open babel software. File conversion had to be done for the molecules to be acceptable in SYBYL2.0® software which was used for preparation of the ligands.

Assessment of Binding: Prior to docking, the ligand molecules have to be prepped as they may contain missing side chain atoms and added water molecules. The SYBYL2.0® software was used in the preparation of the ligand molecules for docking. Energy minimization was also done for all the ligand molecules. The target molecule was then prepared for docking by removal of water molecules and any added molecules if present. Charges were then added to bring the protein to a stable condition.

Molecular docking was then performed between the ligand molecule and the target using SYBYL2.0® software. The grid parameter and docking parameters were inputted and the docking results analyzed.

Results: The chemical structure of the chosen ligands used is tabulated in Table 1: The chemical structure of the target molecule is represented by Figure 1.

Table 1: chemical properities of active components of licorice

S.N.	Molecule	Pubchem id	Molecular weight	Hydrogen bond acceptor	Hydrogen bond donor	Chemical structure
1.	Glycyrrhizin	14982	822.9 g/mol	16	8	
2.	Glabrene	480774	322.4 g/mol	4	2	
3.	Glabridin	124052	324.4 g/mol	4	2	

Molecular docking of the ligand molecules was done with the TGF-β Receptor 2 molecule and the scores obtained were tabulated. The total score refers to the binding affinity of the molecule and is represented in log value. Crash data refers to the abnormal penetration of the ligand into the target molecule while the polar value represents the information regarding the hydrogen bonding and salt bridge interactions. The G score represents the free energy of binding. The scores for molecule are tabulated in table 2.

Table 2: Binding affinity of active compounds in licorice and TGF-β receptor 2 molecule

S.N.	Molecule	Total score	G score	Polar score	Crash score	Hydrogen bonds formed
1.	Glycyrrhizin	2.2400	-147.9896	1.0200	-0.8300	1
2.	Glabrene	3.0400	-116.3689	2.0700	-2.1100	4
3.	Glabridin	2.2700	-114.0368	1.1500	-1.8500	1

Following the docking procedure, Glabrene showed the greatest binding affinity for TGF-β receptor type 2 (Figure 4) when compared to the other ligand molecules. A single hydrogen bond was formed for Glycyrrhizin (Figure 2) and glabridin (Figure 3) with the TGF-β type 2 receptor while four hydrogen bonds were formed between glabrene and the TGF-β type 2 receptor molecule signifying strong union formed between the ligand and the active kinase domains of TGF-β.

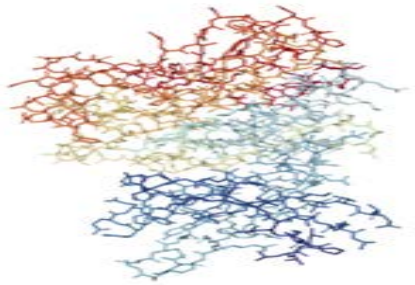


Figure 1: Tgf Beta Molecule

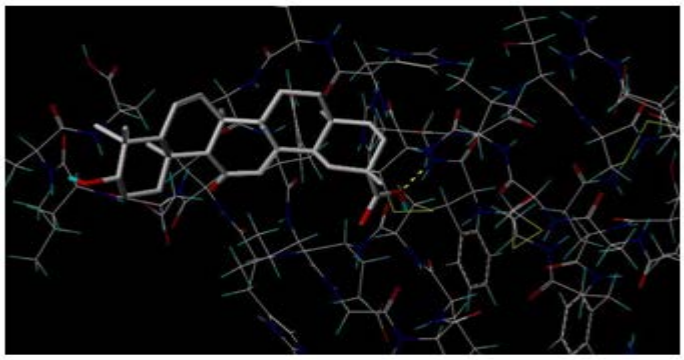


Figure 2: Glycerrhizin Bound To Tgf Beta

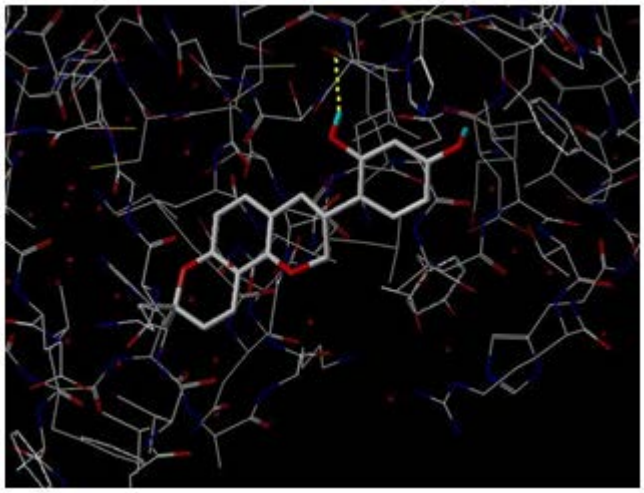


Figure 3: Glabridin Bound To Tgf Beta

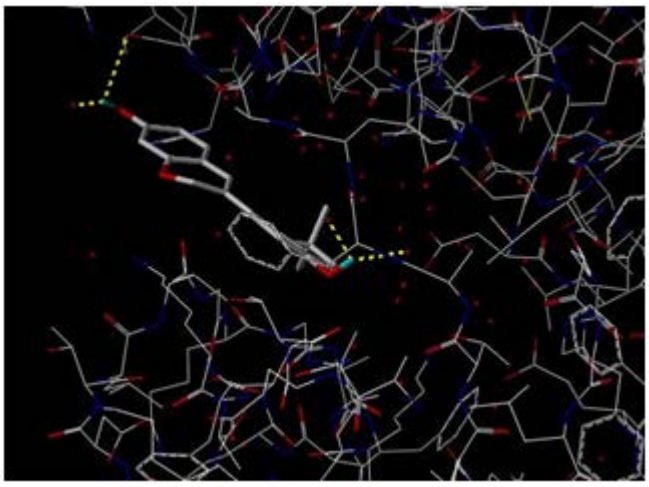


Figure 4: Glabrene Bound To Tgf Beta

Discussion

OSMF is a potentially malignant disorder with a high rate of malignant transformation, especially within the Indian subcontinent where tobacco chewing is commonly prevalent. Chronic areca nut chewing incites a chronic inflammatory response, which results in the recruitment of inflammatory cells to the site of injury. Inflammation induces the production of inflammatory mediators and growth factors like TGF-β which begins a cascade of events through activation of numerous genes to transform fibroblasts into myofibroblast and in turn bring about deposition of excessive collagen matrix[5].

The current treatment modalities used in the treatment of OSMF include physiotherapy, use of enzymes and steroids or surgical excision of the bands[6]. Though various treatment modalities have been tried none have been curative so far. Hence, the attention has turned towards ayurvedic and herbal remedies for finding a better cure[7], [8].

Licorice is one such medicinal herb used in traditional medicine. Licorice is a commonly used herb for various ailments such as sore throat, peptic ulcers, psoriasis etc. due to its anti- inflammatory and antibacterial properties[9], [10]. Studies have identified numerous active ingredients in licorice including Glycyrrhizin, and isoflavanoids like Glabrene and glabridin. Numerous in-vitro studies have shown the potential antifibrotic action of licorice in hepatic and lung fibrosis models[11], [12]. Studies done on bleomycin-induced lung fibrosis show that licorice decreases the expression of inflammatory mediators like IL-1β, TNF-α and reduced collagen deposition by interfering with TGF-β1[11], [12]. In a study done by Lee et al, glabridin suppressed the expression of TGF-β as well as the expression of phosphor-Smad2 in fibrotic buccal fibroblasts[13]. Though studies suggest an antifibrotic potential for licorice, its exact mechanism of action has not been elucidated at the molecular level.

Bioinformatics is a field of study that combines multiple arenas of research including biology, information engineering, statistics, and mathematics to examine biological data. Molecular docking is a virtual screening method used to assess the binding affinity of a ligand with a particular target molecule. This technique aids in the recognition of the binding site on the target molecule and helps to assess the potential pharmacological effects of the ligand.

In a molecular docking study by KSandagalla et al, binding efficiency of TGFβR 1 and curcumin was assessed. Six curcumin analouges which inhibited ALK5 receptor was identified through this study and they proposed that curcumin could be used as therapeutic agent for the treatment of malignancies[14].

In the current study we assessed the binding affinity of the active compounds of licorice and TGF-β receptor. Our results showed that glabrene formed strong hydrogen bonding with TGF-β receptor molecule proving the anti-fibrotic potential of the compound at the molecular level. The other active ligand molecules also formed bonding with the target molecule. This suggests that licorice could be used as potential antifibrotic drug that directly inhibits TGF-β, thereby halting the fibrotic cascade initiated in OSMF.

Our study has certain limitations. Since the study was based on virtual docking the confounding factors that could act at the in vivo level could not be assessed. The study was also limited to a single herbal compound and a single protein target. Future studies should be done to assess the active compound of licorice with other protein targets involved in the fibrotic cascade to further understand the therapeutic potential of licorice.

In the current era, the need of the hour is the unearthing of newer drugs and targeted therapies to treat illnesses. Our study suggests that licorice could be used as a potential antifibrotic drug substitute, to the commercially available drugs loaded with adverse effects.

Conclusion

In silico pharmacomodelling has become increasing popular over the past decade for designing newer drugs. Commercially available drugs are often loaded with side effects and hence attention has shifted towards plant derived phytochemicals. Our study confirmed that glabrene docks well with TGF-β suggesting the role of glabrene in inhibiting TGF-β mediated fibrosis.

Further in-vitro studies are however required to assess if they inhibit TGF-β and subsequent fibrosis in induced fibrosis models.

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