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MOLECULAR DOCKING ANALYSIS OF GLABRIDIN AND DEXAMETHASONE FOR TARGETING INFLAMMATORY PATHWAYS IN ORAL SUBMUCOUS FIBROSIS

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Abstract:
The study aimed to evaluate the anti inflammatory potential of Glabridin, a phytochemical found in Licorice, by virtually assessing its binding efficacy on multiple protein surfaces using molecular docking. The X-ray crystallographic structure of the target proteins was downloaded from the Protein Data Bank, and ligand preparation was done using Ligprep. Molecular docking was performed using the Glide algorithm, and all results were analyzed using Glide Score. The study found that Glabridin significantly interacted with all the target proteins, with docking scores ranging from -4 to -10. The findings of this study suggest that Glabridin could potentially be used to treat OSMF due to its anti-inflammatory property. However, further studies are needed to validate these results in vitro and in vivo.

Introduction

Oral Submucous fibrosis (OSMF) is a potentially malignant disease described by Schwartz in 1952 as “Atropica Idiopathica mucosae Oris” and later by Jens J Pindborg in 1966 as “an insidious chronic disease of any part of the oral cavity, sometimes affecting the pharynx” (1). OSMF is also characterised by reduced movement of tongue and papillae, whitening and leathery texture of oral mucosa , progressive decrease in mouth opening, and narrowing of uvula (1). The prevalence of OSMF in India is estimated at 0.2-2.3% in males and 1.2-4.6% in females, with a wide range of 11-60 years. Significant increases in incidence has been observed following widespread distribution of commercial tobacco and betel nut products sold in single use packages, commonly referred to as Gutkha. Currently, an estimated 10-20% of worlds population consumes various formulations of betel nut (1).

Interventions for the treatment of OSMF include a wide range of drugs, including dietary supplements (vitamins and antioxidants), antiinflammatory agents (corticosteroids), proteolytic agents (such as hyaluronidase and placental extracts), vasodilators, immune regulators and anticytokines. These can be administered orally, topically or by submucosal injection. Advanced cases of OSMF are treated surgically [2]. Along with these treatments other allied therapies including Ayurvedic, Homeopathic and physiotherapy can also be used as added modalities in the conventional present treatment that is safe and effective specifically in country like India [3].

Phytochemicals are gaining popularity as a treatment for OSMF due to comparatively lesser range of side effects . Licorice is an example of a perennial plant of which Glabridin has multiple properties like antioxidant, antiatherogenic, anti inflammatory, anti tumour, antifibrogenic. Licorice is native to middle east, southern Europe and India. It is a commonly used sweetener in food industry. In India, Ayurveda has long been using licorice contents to treat ailments. Numerous studies have been conducted to evaluate the anti-fibrotic properties of licorice in models of liver and kidney fibrosis.
Molecular docking is a subset of bioinformatics used to study the relationships and interactions between two or more molecules at the molecular level. It is used to predict the 3dimensional configuration of any complex formed when a ligand binds to a specific target molecule. Molecular docking not only helps characterise the behaviour of ligand molecules , but also helps identify binding sites on target molecules, which help elucidate the fundamental biochemical processes involved (4)
The current study is aimed to virtually evaluate the binding efficacy of Glabridin found in Licorice on inflammatory protein receptors by molecular docking to assess the anti inflammatory potential of it.

Materials and Methods

Xray crystallographic structure of all target proteins were downloaded from Protein Data Bank (PDB). PDB id of the proteins listed in Table 1. All the protein structures were prepared using `Protein preparation wizard' workflow in Schrodinger suite. This involved addition of hydrogen atoms to protein, assigning of bond orders and deletion of unnecessary water molecules around 4Angstroms in active site. Side chains were added, disulphide bonds were formed, missing atoms were added and the partial charges were assigned. Energy minimisation was done using OPLS_2005 (Optimised potentials for Liquid Simulations) force field. Among the downloaded proteins, except NFK-β and TNF-α others were co-crystallised structures and so the ligand binding sites were used so as to define active site of the protein. Receptor grid generation workflow was used to define a grid box around the ligand, to keep all functional residues in grid.

Binding site analysis:

For NFK-β and TNF-α, Sitemap tool from Schrodinger was used to perform for binding site identification and evaluation. Sitemap generates site scores to effectively distinguish sites that can bind and that cannot. Sitemap with highest site score was selected as the potential binding site.

Ligand preparation:

Based on literature review, Glabridin was selected and its 3D structure was downloaded from Pubchem. Using Ligprep, pre-processing of the ligands were done which includes formation of tautomers and ionisation states at pH at 7.0± 2.0 using Epik , hydrogen atoms were added, charged groups were neutralised and geometry of the ligands were optimised.

Molecular docking:

Glide Algorithm was used (glide based ligand docking with energetic) along with extra precision docking for the prepared ligands and proteins. Structure of the ligand was kept flexible to generate different conformations. OPLS force fields were used to perform these calculations. All glide docking runs were performed on CPU specifications of 2.3GHz, 8gb RAM, Linux operating system. All results were analysed using Glide Score.

Results

It was found that glabridin had significantly interacted with all the target proteins. Docking scores were found to be in the range of -4 to -10. Table 2 shows docking scores of glabridin with target proteins and standard inhibitors. Table 3 shows binding energies in the form of glide energy, electrostatic bonding, Van der Waals, hydrogen bonding, residues and bond length.

It was observed that docking scores of standard inhibitor and glabridin were comparable and that the co-crystallised ligands were almost superimposing with the respective docked conformation of ligands. Table 2 represents Glabridin with COX-2, the docking score obtained was -7.82 while that of standard inhibitors such as Celecoxib, Aspirin, Diclofenac, Nimesulide, Naproxen, Ibuprofen displayed the following docking scores -9.713, -7.193, -10.381, -6.79, -8.362, -8.335 respectively. Glabridin forms hydrogen bonding with Arg120; pi-pi stacking with Arg120, Tyr355.
Docking score of -5.552 was obtained on the interaction of glabridin with PLA2. Glabridin exhibits hydrogen bonding with Gly 28 and Asp 47 residues, pi-pi stacking with Hid 46.
Glabridin on interaction with IRAK-4 showed a docking score of -7.605. Hydrogen bonding was observed between glabridin and Asp 272, Asp 278.
Glabridin showed a docking score of -7.529 with Prostacyclin synthase. Hydrogen bonding with Pro433.
With 5-LOX, glabridin showed docking score of -4.55. , hydrogen bonding was observed with Gln 363and Phe 177 , a pi-pi stacking with Phe 177.
Glabridin showed a docking score of -6.08 with IL-β. Hydrogen bonding was observed with Glu B:33 and Cys B:27.
In case of Glabridin with NFK-β, a dock score of -4.651 was obtained. hydrogen bonding with Lys A:98, Glu A:116, Pro A:100. Pi-Pi stacking has been formed with Arg C:103.
Glabridin exhibited a docking score of -5.811 with TNF-α, . Hydrogen bonding with Glu E:116 and Lys D:98, Pro D:100 has been present.
Comparison of Glabridin with standard drug for OSMF dexamethasone is also done by molecular docking. Protein Data Bank ids of protein molecules is same as that seen in table 1.
As seen in Table 4 in case of glabridin and COX-2 a docking score of -7.82 is observed compared to dexamethasone showing docking score of -7.213. In case of glabridin and PLA2 a docking score of -5.552 is observed compared to dexamethasone showing docking score of -6.289. In case of glabridin and IRAK-4 a docking score of -7.605 is observed compared to dexamethasone showing docking score of -6.015. In case of glabridin and Prostacycline Synthase a docking score of -7.529 is observed compared to dexamethasone showing docking score of -7.453. In case of glabridin and 5-LOX a docking score of -4.55 is observed compared to dexamethasone showing docking score of -6.372. In case of glabridin and IL-1β a docking score of -6.08 is observed compared to dexamethasone showing docking score of -6.15. In case of glabridin and NFK-β a docking score of -4.651 is observed compared to dexamethasone showing docking score of -4.542. In case of glabridin and TNF-α a docking score of -5.811 is observed compared to dexamethasone showing docking score of -5.595. In case of glabridin and 3B6H a docking score of -5.012 is observed compared to dexamethasone showing docking score of -5.257.
Figures 1-8 represent the three dimensional molecular binding confirmations of Glabridin with respective protein molecules.

DISCUSSION

Study done by C V Chandrashekar et al have explored the anti-inflammatory mechanism on COX and LOX products by the phytoconstituents contained in G.glabra. It was evident from the research that Glabridin exhibited inhibition of prostaglandin E2, COX and LOX products. Also, the isoliquiritigenin component exerted inhibitory action against COX but the glycyrrhizhin component failed to exhibit any of inhibitory actions. The extended knowledge from the research findings on the underlying molecular mechanisms can be implemented and to improve treatment outcome of various inflammatory disorders at initial stages. This is similar to what we explored and concluded in our study. [5]
Rina et al carried out a study to investigate (based on anti-inflammatory properties) the interaction methods of specific natural compounds using molecular docking analysis. The targets were Phospholipase A2, Cox-2, Interleukin-1 receptor and NFK-β inhibitor. Based on molecular docking

analysis, flavanoids were found to have greater affinity and consistency towards macromolecular targets. This was similar to what we found in our study. [6]

In this research study conducted by Halim et al, a docking analysis was carried out on five commercially available drugs targeted at IL-1β/IL-1R1 protein interface. The site A and site B of interleukin were docked individually. The analysis showed IL-1β site A had more interactions than IL-1R1 chain. In contrary, IL-1R1 chain site B had more interactions than IL-1β. The authors concluded that Site A contains vital amino acids which is required for blockage and binding of Il-1β/IL-1R1 and these sites to be targeted in developing more specific inhibitors. Research based on structure drug discovery, targeted against the interleukin can be designed based on the findings of the study which was similar to our research study. [7]

The relation between chronic inflammation and neoplasms have been reported since decades. Tumour Necrosis factor alpha (TNF-α) is largely associated with anti-tumour activity but now, it's association as mediators of inflammation is reported. TNF-α induces and mediates numerous other inflammatory chemical mediators. Gautam et al in this article have discussed the comprehensive role of TNF-α, in every stage of tumour development and its action as a major switch in establishing a possible link between inflammation and neoplasm which we also found in our study. [8]

The main objective of the research study conducted by Chung-Hung et al was to examine Cox-2 expression up-regulation within Oral Submucous fibrosis affected tissues and also to further evaluate various possibility which might lead to upregulation expression of Cox-2 molecule (in-vivo). The findings of the study revealed there was marked up-regulation of Cox-2 in submucous fibrosis specimens. Also it was found that Arecoline along with constituents of arecanut is chiefly responsible for up-regulation which we also found in our study. [9]

The research conducted by Leena et al was designed with chief objective to evaluate the efficacy of dexamethasone and hyaluronidase as a therapeutic drug combination in treatment of clinically diagnosed Grade 3 Submucous fibrosis. Patients were administered with dexamethasone 1.5 ml along with enzyme – Hyaluronidase, dosage of 1500 IU with 0.5ml solution of lignocaine HCL. This drug combination was injected into Submucous fibrosis affected tissue at time interval of twice a week continued for 4 weeks. There was a significant improvement in the mouth opening suggesting elimination of surgical management of the disease which was similar to our study. [10]

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TABLE 1

Molecule	PDB id
Cyclo-oxygenase 2	5IKV
Phospholipase A2	5G3M
Interleukin Receptor Associated Kinase-4	2NRU
Prostacyclin synthase	3B6H
5-LOX	3V99
Interleukin-1β	1ITB
Nuclear factor Kappa beta	1IKN
Tumour Necrosis Factor alpha	5M2I

TABLE 2

Target Protein		Docking Score
Cox-2	Glabridin	-7.82
	Diclofenac	-10.381
	Celecoxib	-9.713
	Naproxen	-8.362
	Ibuprofen	-8.335
	Aspirin	-7.193
	Nimesulide	-6.79
PLA2	Glabridin	-5.552
	Anagrilide	-5.355
IRAK-4	Glabridin	-7.605
	Inhibitor	-9.029
Prostacyclin synthase	Glabridin	-7.529
	Indomethacin	-8.054
5-LOX	Glabridin	-4.55
	Zileuton	-4.424
IL-1β	Glabridin	-6.08
	Standard Inhibitor	-6.684
NFK-β	Glabridin	-4.651
	Bortezomib	-5.694
TNF-α	Glabridin	-5.811
	Standard Inhibitor	-5.535

TABLE 3

Target	Drug name	Glide energy(kcal/mol)	δG_{edw} (kcal/mol)	δG_{ecol} (kcal/mol)	Xphbond (kcal/mol)	Interacting residues/molecules
Cox-2	Glabridin*	-3.358	1.01	-4.368	-0.386	Tyr 355 Arg 120
	Diclofenac#	-41.945	-33.108	-8.837	-1.222	Arg 120 Ser 530
	Celecoxib#	-12.721	-1.812	-10.909	0	Tyr 355 Arg 513 Ser 353 Gln 192 Phe 518
	Naproxen#	-32.98	-28.831	-4.149	-0.96	Tyr 355
	Ibuprofen#	-26.286	-22.841	-3.446	-0.67	Tyr 355
	Aspirin#	-29.564	-24.611	-4.953	-1.588	Tyr 385 Ser 530
	Nimesulide#	-18.875	-19.079	0.205	0	Tyr 355
PLA2	Glabridin*	-40.071	-32.265	-7.805	-0.35	Hid 46 Asp 47 Gly 28
	Anagrilide#	-32.025	-32.863	0.838	0	Gly 28
IRAK-4	Glabridin*	-44.51	-37.46	-7.05	-0.663	Asp 272 Asp 278
	Inhibitor#	-57.62	-51.79	-5.831	-0.7	Met 265 Lys 213 Tyr 262
Prostacyclin synthase	Glabridin*	-45.999	-40.587	-5.412	-0.7	Pro 433
	Indomethacin#	-48.695	-43.921	-4.774	-0.7	Leu 442
5-LOX	GLabriidn*	-35.077	-28.28	-6.797	-0.535	Gln 363

						Phe 177
	Zileuton#	-32.599	-25.648	-6.951	-1.33	Gln 363
Interleuki-1β	Glabridin*	-8.66	-4.253	-4.407	-0.35	Glu B:33 Cys B:27
	Inhibitor#	-38.496	-32.538	-5.957	-2.708	Glu B:10 Asn B:30 Ser A:21
NFK-β	Glabridin*	-41.062	-31.019	-10.043	-0.918	Arg C:103 Glu A:116 Lys A:98 Pro A:100
	Bortezomib#	-55.153	-41.436	-13.717	-1.766	Gln C:102 Glu A:116 Arg C:103
TNF-α	Glabridin*	-44.525	-32.914	-11.612	-1.613	Glu E:116 Lys D:98 Pro D:100
	Inhibitor#	-51.65	-45.892	-5.758	-0.089	Arg F:103 Gln F:102

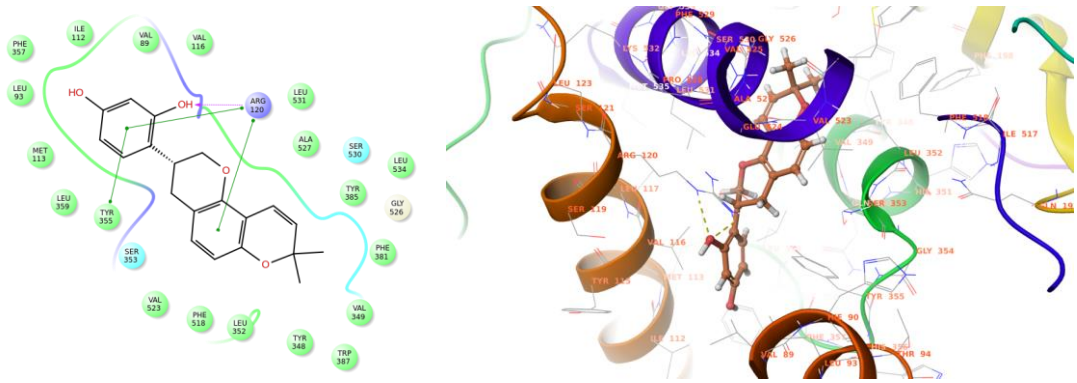

FIGURE 1: Molecular interactions of glabridin with COX-2

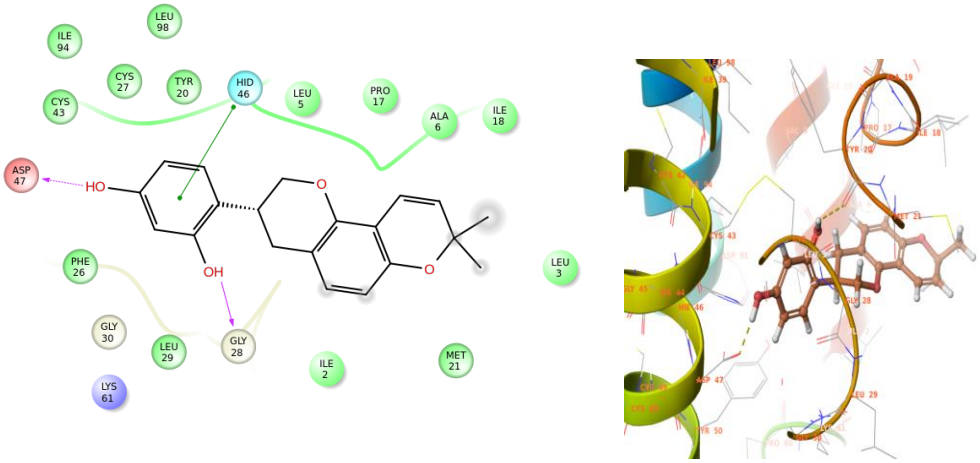

FIGURE 2: Molecular interactions of Glabridin with Phospholipase A2 (PLA2

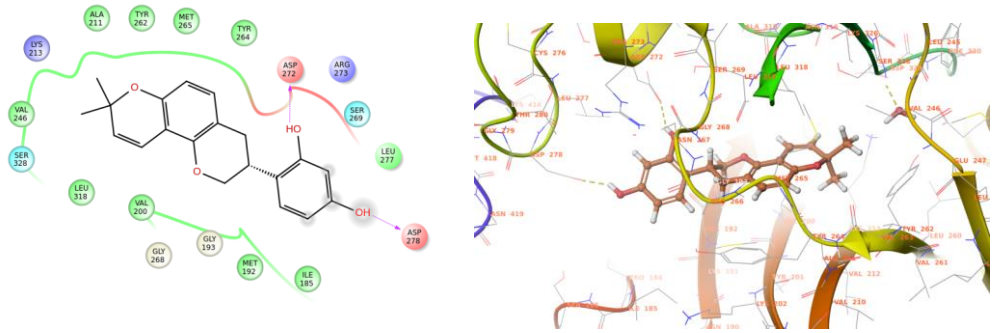

FIGURE 3: Molecular interactions of Glabridin with Interleukin Receptor Associated Kinase 4 (IRAK-4):

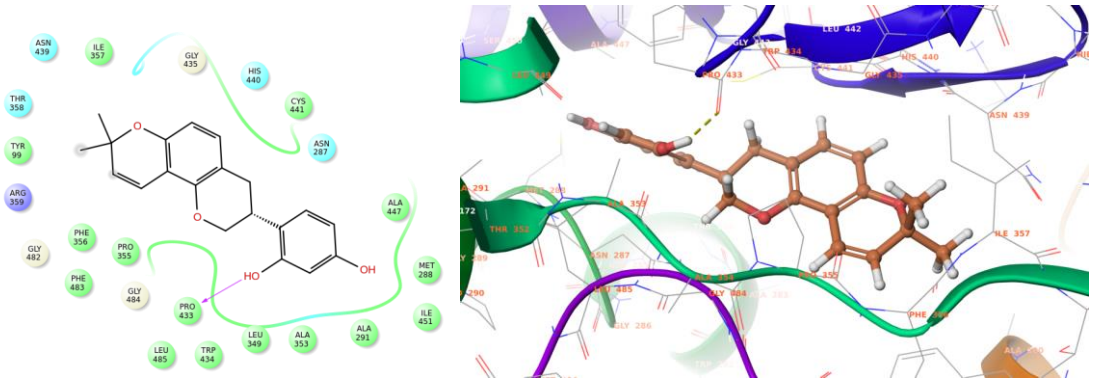

FIGURE 5: Molecular interactions of Glabridin with 5-Lipooxygenase (LOX)

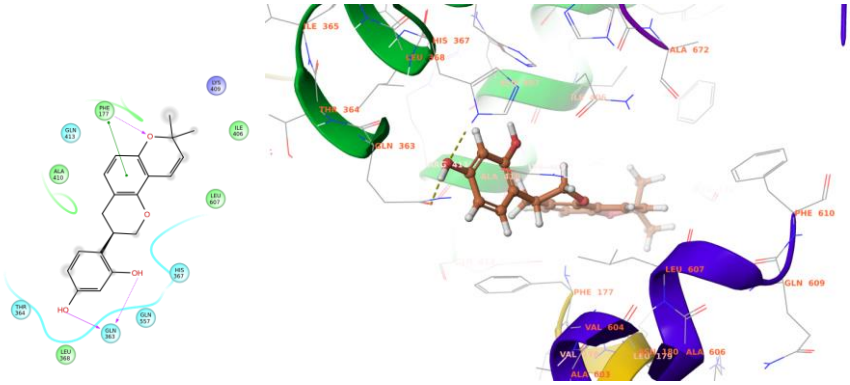

FIGURE 6: Molecular interactions of Glabridin with Interleukin-1β (IL-β):

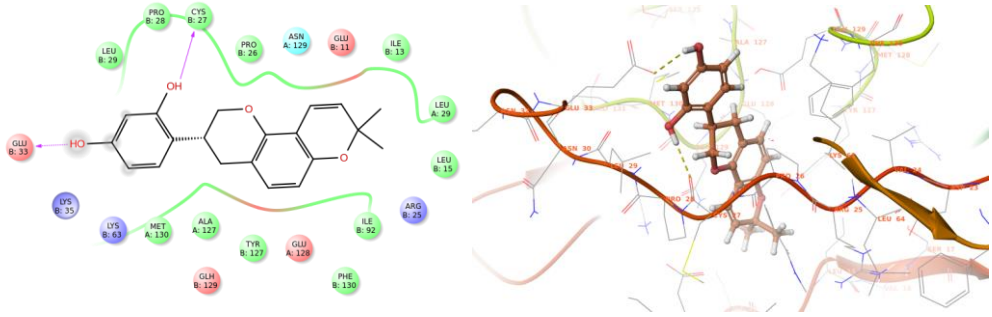

FIGURE 7: Molecular interactions of Glabridin with Nuclear Factor Kappa Beta (NFK-β):

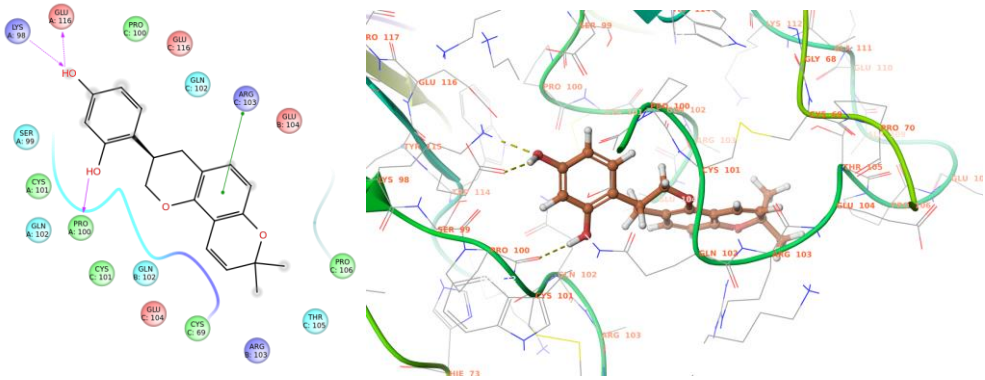

FIGURE 8: Molecular interactions of Glabridin with Tumour Necrosis Factor Alpha (TNF-α):

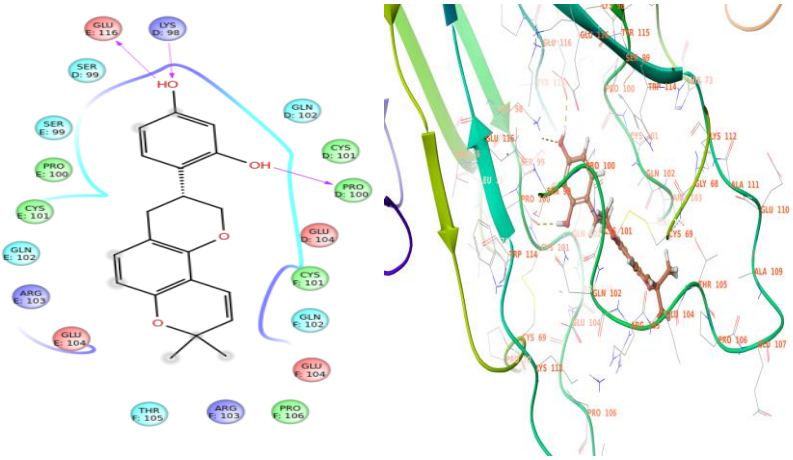

TABLE 4: Dock Score of Glabridin and dexamethasone with multiple anti-inflammatory target proteins

Target Protein		Docking Score
Cox-2	Glabridin	-7.82
	Dexamethasone	-7.213
PLA2	Glabridin	-5.552
	Dexamethasone	-6.289
IRAK-4	Glabridin	-7.605
	Dexamethasone	-6.015
Prostacyclin synthase	Glabridin	-7.529
	Dexamethasone	-7.453
5-LOX	Glabridin	-4.55
	Dexamethasone	-6.372
IL-1β	Glabridin	-6.08
	Dexamethasone	-6.15
NFK-β	Glabridin	-4.651
	Dexamethasone	-4.542
TNF-α	Glabridin	-5.811
	Dexamethasone	-5.595
3B6H	Glabridin	-5.012
	Dexamethasone	-5.257

References

1. Rao NR, Villa A, More CB, Jayasinghe RD, Kerr AR, Johnson NW. Oral submucous fibrosis: a contemporary narrative review with a proposed inter-professional approach for an early diagnosis and clinical management. J Otolaryngol Head Neck Surg. 2020;49(1):3

2. More CB, Jatti Patil D, Rao NR. Medicinal management of oral submucous fibrosis in the past decade- A systematic review. J Oral Biol Craniofac Res. 2020;10(4):552–68.
3. Gaikwad P, Iyer J, Fulsundar P, Darak R. A review of various allied therapies for treatment of oral Submucous Fibrosis. 2018
4. Amritha James, Dr. Ramya R., Ramya. M, Preethi Arunachalam, Lekshmy Jayan, "Study of Binding Modes of Active Ingredient of Licorice on TGF-β receptor: A Molecular Docking Approach", IJDSIR- March - 2020, Vol. – 3, Issue -2, P. No. 73 -78.
5. C.V. Chandrasekaran , H.B. Deepak, P. Thiyagarajan, S. Kathiresan, Gopal Krishna Sangli, M. Deepak, Amit Agarwal (2011) Dual inhibitory effect of Glycyrrhiza glabra (GutGardTM) on COX and LOX products, Phytomedicine, 18, pp.278-284
6. Rina Herowati and Gunawan Pamudji Widodo (2017) Molecular Docking Analysis: Interaction Studies of Natural Compounds to Anti-inflammatory Targets, Quantitative Structure-activity Relationship, IntechOpen.
7. Halim SA, Jawad M (2015) Attempt to Explore the Binding Mechanism of IL-1β Inhibitors via Molecular Docking Studies, Med chem 5,pp. 452-457.
8. Gautam Sethi, Bokyung Sung, Bharat B. Aggarwal (2008), TNF: A master switch for inflammation to cancer, Frontiers in Bioscience, pp 5094- 5107.
9. Chung-Hung Tsai, Ming-Yung Chou, Yu-Chao Chang (2003), The up-regulation of cyclooxygenase-2 expression in human buccal mucosal fibroblasts by arecoline: a possible role in the pathogenesis of OralSubmucousFibrosis, J Oral Pathol Med, 32, pp.156-153.
10. Leena James , Akshay Shetty , Diljith Rishi , Marin Abraham (2015), Management of Oral Submucous Fibrosis with Injection of Hyaluronidase and Dexamethasone in Grade 3 Oral Submucous Fibrosis: A Retrospective Study, Journal of International Oral Health, 7(8), pp.82-85
11. Leena James , Akshay Shetty , Diljith Rishi , Marin Abraham (2015), Management of Oral Submucous Fibrosis with Injection of Hyaluronidase and Dexamethasone in Grade 3 Oral Submucous Fibrosis: A Retrospective Study, Journal of International Oral Health, 7(8), pp.82-85