Texila International Journal of Public Health
Special Issue-1 2024
DOI: 10.21522/TIJPH.2013.SE.24.01.Art009

The Effect of Aspalathin on SMAD2, SMAD3, TGF-β - A Major Contributor of Inflammation – An *In-silico* Approach

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Abstract

Chronic inflammation plays a crucial role in the development of several illnesses, and the transforming growth factor-beta (TGF-β) pathway, including SMAD2 and SMAD3, regulates inflammatory responses. Aspalathin, a naturally-occurring compound extracted from several plant sources, has shown potential anti-inflammatory properties. This study uses computational methods to investigate how aspalathin affects SMAD2, SMAD3, and TGF-β in order to understand how it regulates inflammation. We use molecular docking to analyze how aspalathin binds with important components of the TGF-β pathway. Our research reveals promising information about the potential of aspalathin, which shows a strong ability to bind with the inflammatory regulator (SMAD2, SMAD3, and TGF-β). Aspalathin may offer therapeutic benefits for treating inflammatory diseases. Further testing in both controlled laboratory environments (in vitro) and inside live creatures (in vivo) are required to validate the computational findings and prove aspalathin's potential as a viable option for inflammation management.

Keywords: *Aspalathin, Inflammation, Molecular Docking, Novel Technique, Smad Proteins, TGF-β.*

Introduction

Aspalathus linearis contains a significant amount of aspalathin, which is a C-glucosyl dihydrochalcone. The distinctive plant life of the Cape Floristic area of South Africa is often used to produce rooibos, a kind of herbal tea. Aspalathin, a flavonoid, was first identified for its role in the reddish-brown hue of fermented rooibos tea. Increased interest in aspalathin's antioxidant qualities has grown due to the emphasis on natural antioxidants and their effectiveness in fighting oxidative stress. This has increased interest in its ability to enhance metabolic syndrome, a condition strongly linked to oxidative stress. Furthermore, aspalathin has potent anti-inflammatory characteristics [1]. Inflammation is a multifaceted and extended process involving the activation, recruitment, and operation of both innate and adaptive immune system cells. The primary acknowledgment of inflammation stemmed largely from its crucial function in the host's immunological response to pathogens. Inflammation is also essential for tissue restructuring, regeneration, and healing. Furthermore, subtle types of inflammation play a crucial role in preserving tissue balance [2]. Recent studies indicate that certain compounds found in rooibos, such as aspalathin and phenylpyruvic acid-2-O-β-d-glucoside (PPAG), have the ability to shield cardiomyocytes against reactive oxygen species (ROS) associated with elevated blood sugar levels. Aspalathin is notably more efficient in this regard since it enhances the body's inherent antioxidant defenses [3]. Inflammation may be categorized according to whether it is caused by an outside chemical or

Received: 22.2.2024 ***Accepted: 11.03.2024*** ***Published on: 29.04.2024***
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an aberrant internal response. Inflammation may seem as either chronic or acute, depending on how long it lasts. Acute inflammation is considered a defensive reaction of the innate immune system that is activated by infection or damage. However, chronic inflammation may develop in some clinical conditions, such as obesity, even without infection or damage [4]. Diseases including cancer, cardiovascular disease [5], diabetes [6–8], and asthma are significantly impacted by inflammation. Diet may affect several stages of inflammation, hence influencing specific inflammatory diseases. Recent studies indicate that polyphenolic chemicals, such flavonoids, present in vegetables, legumes, fruits, and cocoa, may have anti-inflammatory effects [9,10]. Recent research has shown that flavonoids may hinder transcription factors or regulatory enzymes that are crucial in modulating inflammatory mediators. Flavonoids, powerful antioxidants, are recognized for their ability to decrease tissue fibrosis or injury [11].

TGF-β has a vital role in immune response, extracellular matrix synthesis, and cell proliferation and specialization [12]. Members of the TGF superfamily vary in their R-Smad activation characteristics. The three TGF isoforms mostly activate Smad2 and Smad3, while they phosphorylate Smad1, Smad5, and Smad8. Smad2 and Smad3 are often seen as interchangeable elements of the TGF-β signaling cascade due to their similar structure and molecular traits. Multiple in vitro studies indicate that Smad2 and Smad3 may have different effects on cellular reactions [13]. TGF-1β is a key mediator in the pathogenesis and triggers the activation of Smad proteins, particularly Smad3. Upon activation by TGF-β, Smad3 and other stress molecules such as Ang II, AGEs, and CRP go to the nucleus and bind to DNA sequences to control the expression of target genes [14]. Molecular docking is a computer technique used to predict the binding affinities and binding modes of a complex formed by many component molecules with known structure [8].

Materials and Method

Protein Target Preparation

The protein targets (SMAD2, SMAD3, and TGF-β) were obtained from the Protein Data Bank (PDB). The protein was created using the Discovery Studio Visualizer 2020. The water molecules included inside the protein molecules were examined and eliminated if necessary. Moreover, the ligands and ions that were connected were removed. Usually, PDB proteins do not include hydrogen atoms. Hydrogen atoms were incorporated into the protein to transform it into a regular protein. Hydrogen atoms are also used in docking research [15]. The protein synthesis was achieved by using optimization and minimization techniques [16,17].

Ligand Preparation

Aspalathin was acquired from the PubChem database. The Discovery Studio Visualizer 2020 was used to create the compounds, and modifications were made to ensure the ligand's isomer with the least energy. Following energy reduction, the ligand molecules underwent molecular docking experiments [18].

Molecular Docking

The produced protein was brought into the laboratory for the experiment. The grid size was set at about 60 angstroms to cover all active site residues identified on the site map. The van der Waals radii of the nonpolar atoms in the receptor and ligand were originally set at 0.50 as the default value. The ideal structural position was identified using criteria such as docking score, glide energy, hydrogen bonding, and hydrophobic interactions.

Results

Table 1. A Log Table Which Shows Affinity and RMSD Value of SMAD2 with Aspalathin

Mode	Affinity (kcal/mol)	Dist from best mode rmsd l.b.	rmsd u.b.
1	-9.1	0.000	0.000
2	-9.1	3.047	5.29
3	-9.0	2.572	4.639
4	-8.9	4.454	8.31
5	-8.9	2.669	7.86
6	-8.9	2.834	4.877
7	-8.9	6.383	8.486
8	-8.7	3.174	6.005
9	-8.5	3.59	8.528

Table 2. A Log Table Which Shows Affinity and RMSD Value of SMAD3 with Aspalathin

Mode	Affinity (kcal/mol)	Dist from best mode rmsd l.b.	rmsd u.b.
1	-9.0	0.000	0.000
2	-9.0	2.807	5.434
3	-8.9	7.839	15.473
4	-8.8	1.969	2.849
5	-8.8	1.873	9.844
6	-8.6	17.004	21.641
7	-8.6	7.584	12.378
8	-8.5	10.869	15.881
9	-8.5	7.053	12.821

Table 3. A Log Table Which Shows Affinity and RMSD Value of TGF-β with Aspalathin

Mode	Affinity (kcal/mol)	Dist from best mode rmsd l.b.	rmsd u.b.
1	-6.7	0.000	0.000
2	-6.7	12.824	14.902
3	-6.5	2.66	4.19
4	-6.5	28.946	32.494
5	-6.5	3.331	4.275
6	-6.4	21.139	24.988
7	-6.4	2.334	3.526
8	-6.3	2.847	4.138
9	-6.3	10.086	12.549

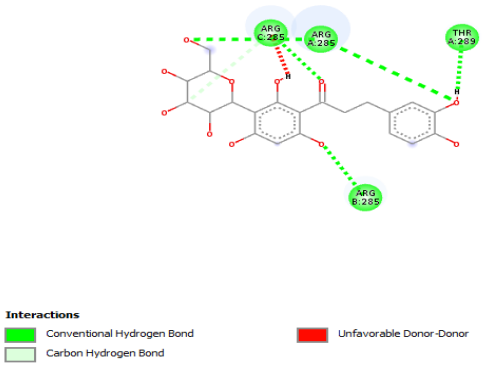

Figure 1. SMAD2 Interacts in 2D Structure with Aspalathin

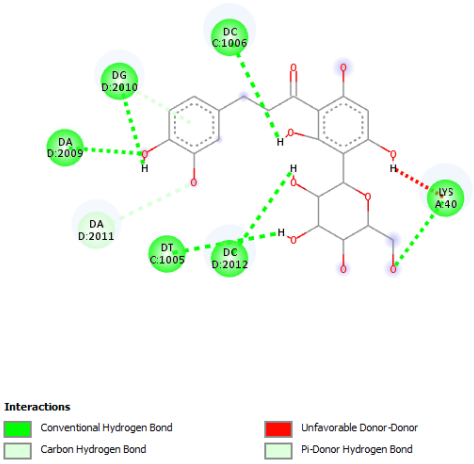

Figure 2. SMAD3 Interacts in 2D Structure with Aspalathin

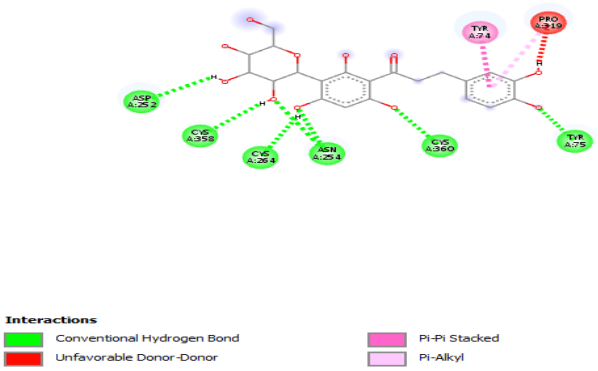

Figure 3. TGF-β Interacts in 2D Structure with Aspalathin

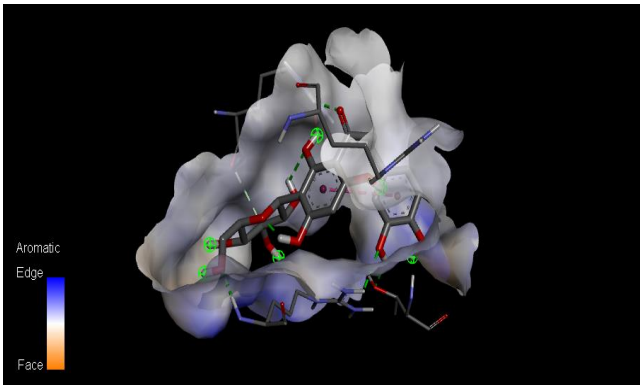

Figure 4. 3D Interaction Between SMAD2 and Aspalathin

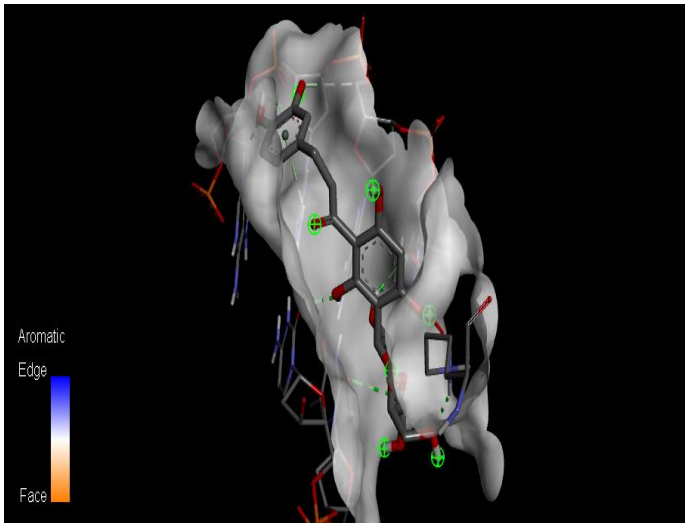

Figure 5. 3D Interaction Between SMAD3 and Aspalathin

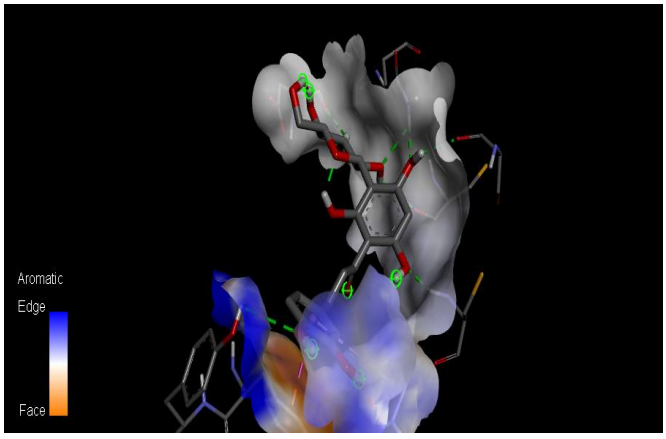

Figure 6. 3D Interaction Between TGF-β and Aspalathin

Table 4. Comparative Analysis of Molecular Docking Outcomes of Aspalathin on SMAD2, SMAD3, and TGF-β

RESULT ANALYSIS	VISUALISATION SOFTWARE	PROTEIN	LIGAND	DOCKING SCORE	AMINO ACID RESIDUE

Auto dock 1.5.7	Discovery Software	SMAD2	Aspalathin	-9.1	Conventional Hydrogen Bond: ARG C:285,ARG A:285, THR A:289,ARG B:285 Carbon Hydrogen Bond: ARG C:285 Unfavourable Donar-Donar: ARG C:285
		SMAD3		-9.0	Conventional Hydrogen Bond: DA D:2009, DG D:2010, DC C: 1006, LYS A:40, DC D:2012, DT C:1005 Carbon Hydrogen Bond: DG D:2010, DA D :2011 Unfavourable Donar-Donar : LYS A:40 Pi-Donor Hydrogen Bond: DA D :2011

		TGF-β		-6.7	Conventional Hydrogen Bond: ASP A:252,CYS A:358, CYS A:264, ASN A:254, CYS A:360, TYR A:75 Unfavourable Donar -Donar : PRO A:319, Pi-Pi stacked: TYR A:74, Pi-Alkyl-PRO A:319

Results and Discussion

Inflammation is essential for the body's immune response to many stressors. Initially, it has a positive impact by stimulating the body's healing mechanisms. Nevertheless, this is worrisome since it might evolve into a self-sustaining cycle, where current inflammation may induce more chronic inflammation [19]. Inflammation is an immune system reaction characterized by swelling, redness, warmth, and pain. It often arises in response to tissue or organ damage. Moreover, it is linked to other medical conditions such as allergies, arthritis, atherosclerosis, and autoimmune illnesses [20].

Table 1 displays the docking analysis results of SMAD2, including the log value representing affinity value and root mean square values. The root mean square value is 0.000, whereas the affinity value is more than -4.5. The chosen docking score was -9.1, accompanied by a substantial root mean square value. The docking score with visualization for 2D and 3D picture structures as shown in fig.1 and fig for SMAD2. The 2D structure displays interactions including conventional hydrogen bonds, carbon-hydrogen bonds, and unfavorable donor-donor interactions. In a molecular docking research, Hepcidin, a hepatic peptide hormone present in the circulatory system, is essential for controlling the body's iron equilibrium. Inflammation leads to elevated hepcidin expression, causing disruption in the body's iron levels. This disturbance is linked to a widespread worldwide condition called anemia of inflammation, which is the second most frequent kind of anemia.

Piperine has shown the ability to interact with proteins such as SMAD1 and STAT3 based on molecular docking studies. The discovered binding patterns indicate that piperine could hinder the activation of proteins that stimulate hepcidin synthesis [21]. In a separate investigation, we found targets linked to Guanxin V using a thorough method that included virtual screening and systematic pharmacology. These targets are especially connected to ventricular remodeling and inflammation. The main results obtained from this comprehensive method were validated by molecular docking investigations using SMAD proteins and in vivo research [22].

Table 2 displays the results of the docking study, including the log value representing affinity and root mean square values of SMAD3. The root mean square value is 0.000, and the affinity value is greater than -4.5. The chosen docking score was -9.0, accompanied by a substantial root mean square value. The docking score is shown in visualization for 2D and 3D picture structures as shown in fig. 2 and fig. 5 for SMAD3, in 2D structure depicting typical carbon-hydrogen bonds, unfavorable donor-donor interactions, and pi-donor bonds. A recent study on persons with type 2 diabetes mellitus (T2DM) found a notable change in the heart's structure known as cardiac fibrosis, which may result in the onset of heart failure (HF). One way to effectively treat cardiac fibrosis is by reducing inflammation via Smad 3 phosphorylation [23].

A comparative analysis of Smad3 in inflammatory renal fibrosis associated with chronic kidney disease (CKD) revealed few viable therapeutic options. Eleutheroside B was shown to bind to Smad3 in molecular docking studies, resulting in a significant decrease in phosphorylated Smad3 (p-Smad3) expression. Eleutheroside B's fibrotic protective effect in HK2 cells was undone by silencing Smad3 [24].

Table 3 displays the results of the docking study, showing the log value representing affinity and root mean square values of TGF-β. The root mean square value is 0.000, whereas the affinity value is more than -4.5.The chosen docking score was -6.7 for TGF-β, accompanied by a notable root mean square value. The docking score is represented visually in 2D and 3D picture structures as seen in fig. 3 and fig. 6. Fig. 3 depicting the 2D structure of TGF-β with typical carbon-hydrogen bonds, unfavorable donor-donor interactions, pi-alkyl interactions, and pi-stacking interactions.

Apigenin, a phytochemical extracted from Chamomilla recutita, has an undetermined function in interstitial cystitis, as shown in a comparable research investigation. Molecular docking research on TGF-β has confirmed apigenin's antioxidant and anti-inflammatory properties [24,25].

Likewise Oral sub-mucous fibrosis (OSMF) is a severe and possibly cancerous disorder that affects the lining of the mouth. Transforming growth factor beta (TGF-β) is a major cytokine linked to cell proliferation, growth, and death. Natural herbal ligands such as Curcumin, Curcumin Pyrazole, and Demethoxycurcumin have strong binding affinity and the highest docking scores for both TGF-β type I and TGF-β type II receptors, as shown by docking results [24–26].

Cervical cancer is a significant cause of cancer-related deaths in women globally, and its epidemiological trend is similar to that of a less contagious sexually transmitted illness. The study results indicate that after completing docking experiments, the chemical nilotinib has substantial potential as an inhibitor of TGF-β1. This inhibition may decrease TGF-β1 expression and halt the development of cervical cancer [27].

The advancement of liver disease is greatly impacted by the development of hepatic fibrosis. E Se tea (ES), a traditional herbal infusion in China, has a wide range of health advantages for humans. The research shown in that ESE (E Se tea extract) has the ability to reduce liver fibrosis. It does this by enhancing antioxidant and anti-inflammatory capacities via the Nrf2/NF-κB pathway and reducing the development of liver fibrosis by blocking the TGF-β/Smad pathway [28].

Chronic kidney disease (CKD) is defined by two main pathological characteristics: inflammation and fibrosis. TGF-β is well acknowledged as a key element in renal fibrosis. TGF-β acts as a strong anti-inflammatory cytokine, playing a role in reducing renal inflammation [29]. Therefore, blocking TGF-β has a double effect in chronic

kidney disease—it impedes renal fibrosis and at the same time stimulates inflammation, demonstrating the complex function of TGF-β in this situation. Abundant evidence has shown that TGF-β1 triggers downstream signaling molecules such as Smad3 and Smad3 [30]. Rheumatoid arthritis, a persistent autoimmune disorder with widespread repercussions, affects around 1% of individuals worldwide. The study investigated the possible therapeutic advantages of theacrine (TC) for arthritis and examined the underlying processes in SD rats produced by Freund's incomplete adjuvant (FIA). The research found that TC had significant anti-arthritic effects via blocking IL-6 and stimulating TGF-β via the TGF-β/SMAD pathway [31-35]. The above results and discussion shows that Aspalathin interacts and alters the SMAD2, SMAD3, TFG-β and it has anti inflammatory effects on these genes (Table 4).

Conclusion

Our computational study on the impact of aspalathin on key elements of the TGF-β signaling cascade, particularly SMAD2 and SMAD3, important factors in inflammation, has shown encouraging results. Our research indicates that aspalathin may have the ability to affect these signaling molecules, perhaps acting as a regulator of TGF-β-induced inflammation. This is an opportunity for further experimental study to confirm our computational findings and explore the therapeutic effects of aspalathin in reducing inflammation linked to TGF-β signaling in different illness scenarios (Represented in fig 7). The queries provide promise for creating new anti-inflammatory methods by using the inherent features of this chemical.

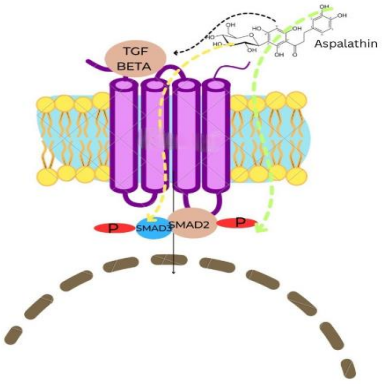

Figure 7. Represents the Interaction of Aspalathin with SMAD2,SMAD3, TGF-β and Alteration of These Genes via Alleviating Inflammation

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors express their gratitude to Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences in Chennai, for their unwavering support, which significantly contributed to the successful outcome of this study.

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