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Original Article

To Evaluate the Affinity of Chlorogenic Acid and Ferulic Acid towards PPAR-Γ and Glutathione S-Transferase Omega-1 Complex with the Docking Simulation and their Pharmacological Activities by Using their Formulation

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ABSTRACT

Objectives: The aim of this study was to perform molecular docking and ADMET analyses of ferulic acid and chlorogenic acid. Through molecular docking, we have observed the receptor affinity, specifically targeting the PPAR-γ receptor for anti-inflammatory activity and the Glutathione S-transferase omega-1 Complex for antioxidant activity. After confirming the affinity of compounds towards these receptor and enzyme, we performed anti-inflammatory and antioxidant activities. **Materials and Methods:** For molecular docking analysis of compounds, PDB ID: 4xum was selected for anti-inflammatory activity and indomethacin was selected as a reference. For the antioxidant activity, ascorbic acid was selected as a reference standard and PDB ID: 3vln was chosen. ADMET studies of these compounds were done by QikProp, SwissADME and ProTox-II software. Anti-inflammatory *in vitro* analysis was performed using protein denaturation and DPPH assay for an *in vitro* antioxidant study. **Results:** Chlorogenic acid gave a capable result as compared to indomethacin and ascorbic acid for both pharmacological activities. The docking scores of chlorogenic acid and ferulic acid for anti-inflammatory activity were -13.236 Kcal/mole and -7.313 Kcal/mole and for antioxidant activity, -7.829 Kcal/mole and -4.758 Kcal/mole respectively. When they were combined in formulation, they gave synergistic effects for both pharmacological activities as compared to their reference standards. **Conclusion:** Ferulic acid and chlorogenic acid formulations have shown the best pharmacological effects for anti-inflammatory and antioxidant activity as compared to marketed formulations. The toxicity study and ADME analysis of both compounds reveal their suitability for pharmacological applications.

Keywords: Ferulic Acid, Chlorogenic Acid, PPAR-Γ, Glutathione S-Transferase Omega-1 Complex, Anti-Inflammatory, Antioxidant.

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INTRODUCTION

Among the significant pharmacological effects that ferulic acid and chlorogenic acid reveal are anti-inflammatory, antimicrobial, antioxidant, anti-cancer and antidiabetic.[1,2] The bioactive molecule ferulic acid has a number of advantages. Antioxidant and anti-inflammatory properties are two of its roles. It suppresses inflammatory responses and efficiently prevents oxidative damage by removing excess Reactive Oxygen Species (ROS), addressing free radicals directly and blocking the enzymes that produce them. It's noteworthy that it is protective for cardiovascular and kidney health.[3,4] Chlorogenic acid exhibits antioxidant activity against free radicals as well as anti-inflammatory activity. This phytochemical comes from the family of hydroxycinnamic, which are phenolic compounds. In many herb, vegetable and fruit-made beverages, this phytochemical is used. To evaluate the antioxidant and anti-inflammatory efficacy of these compounds, many *in vitro* and *in vivo* studies were conducted.[5]

PPAR-γ is a transcription factor protein and it controls inflammation, cell differentiation and the metabolism of fat- and carbohydrate-related genes, which is also called NR1C3. This protein is a member of the nuclear receptor class called PPARs (Peroxisome Proliferator-Activated Receptors). There are three distinct PPARs, namely α, β and γ, each encoded by a separate gene. The process that produces fat cells is called adipogenesis and this protein has a high affinity for adipose tissues throughout the body.[6,7] The anti-inflammatory effect of PPAR-γ exhibits several

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steps, including cytokines and NF-κB suppression, which is an inflammatory transcription factor. Also, by activating a series of cellular reactions, anti-inflammatory M2 macrophages balance shifting PPAR-γ inhibit inflammation, conceivably affecting the activity of inflammatory enzymes. Moreover, PPAR-γ activates Interleukin-10 and stimulates the synthesis of certain chemicals that efficiently suppress inflammation.[8,9]

Naturally occurring toxins and normal metabolic processes produce free radicals, which continuously stress the body's antioxidant defences, which leads to oxidative stress. Also, it causes cellular damage and chronic inflammation, which can set off a chain reaction of pathologies that includes accelerated ageing, compromised cellular function and long-term conditions including diabetes, cancer and heart disease. Glutathione S-transferase omega-1 enzyme helps dangerous compounds and free radicals combine with glutathione.[10,11] Glutathione S-transferase omega-1 enzyme, removes toxins from the body and helps in detoxification. This enzyme belongs to the class of enzymes known as glutathione S-transferase omega-1. This enzyme could be involved in stress-related reactions. It might also affect molecules that cause inflammation by changing the metabolism of various toxins and carcinogens, as well as disease susceptibility. By combining harmful and cancer-causing chemicals with glutathione, it functions as a vital cellular antioxidant. The GSTO1 enzyme neutralises harmful substances and removes them from the body through this method. By removing dangerous compounds from the body, it helps the antioxidant system of cells in an indirect way.[12-14]

Many unfavourable effects of dysregulated or chronic inflammation cause edema, pyrexia, nociception and tissue damage, which ultimately cause functional restrictions and reduced mobility. Managing such chronic inflammation and its effect on life in the context of medication is still a major issue.[15] Chronic inflammation has a role in the pathophysiology and advancement of a number of chronic illnesses, more significantly those that are common in modern medicine, such as atherosclerosis, type II diabetes and several cancers.[16] PPAR-γ agonism provides a potential therapeutic target for anti-inflammatory activity and chlorogenic acid emerged as a possible ligand with documented PPAR-γ activation, which may account for its observed anti-inflammatory activities. Also, the antioxidant properties of ferulic acid and chlorogenic acid might help explain their suggested anti-inflammatory actions by reducing inflammatory cascades mediated by free radicals.[17,18]

The purpose of this study was to determine the ferulic acid and chlorogenic acid-modulated PPAR-γ activity and the function of the glutathione S-transferase omega-1complex. These natural compounds and the previously indicated targets were subjected to a molecular docking simulation-based computational technique to evaluate possible binding interactions. For testing the efficacy of formulated ferulic acid and chlorogenic acid creams, *in vitro* analyses were carried out after the *in silico* analysis. The relative potency of these formulations was then evaluated by comparing their biological activity to reference standards.

MATERIALS AND METHODS

Chemical and reagents

Ferulic acid and chlorogenic acid were procured from Yucca Enterprises, Mumbai, India. Reference standards were purchased from local druggist and chemist shop, while the excipients used in formulation development were purchased from Molychem, Mumbai, India.

Instrumentation and software

Molecular docking investigations were conducted using Schrodinger software (Glide v9.1). Binding free energies were computed using MMGBS/SA. ProTox-II was used to predict toxicity, while QikProp v6.8 and SwissADME were used to compute the ADME characteristics. The cream mixture was homogenised using a homogenizer. The produced creams' spreadability was determined using spreadability. A pH meter for measuring the pH of creams and a stability chamber for investigations on the stability of prepared formulations. To evaluate the anti-inflammatory activity and DPPH antioxidant activity, a UV spectrophotometer was used.

Molecular docking study

The Glide module (Glide v9.1) of the Schrodinger programme was employed in the molecular docking investigations. Then computed the binding free energies using MMGBS/SA. The ADME characteristics were calculated using ADMET Prediction, QikProp v6.8 and SwissADME; the toxicity prediction for the highest scoring hits from the MM-GBSA research was done using ProTox-II (https://tox-new.charite.de/protox_II/). Each of the various methods has been explained independently.

The protein structures that were co-crystallized were sourced from the publicly available Protein Data Bank (PDB ID: 4xum) and (PDB ID: 3vln) at http://www.resb.org. Meanwhile, a selected ligand and reference molecules were utilised for molecular docking studies. The imported protein was then put through a series of processes in the protein preparation wizard, involving the addition of the missing H atom and the proper band order assignment. Formal charges were applied to protein co-factors and an internal ligand heterostate was produced. To remove any unphysical connections among the protein or ligand atoms and preserve the relaxed state of the co-crystallized protein complex, the final stage was performing OPLS 2005 forcefield energy minimization. Additionally, any water molecules within the range of 5 were eliminated. The receptor grid was created to identify the exact site of binding for the molecules being studied. Using a 1.0 scaling factor, default settings were applied and the centroid of the co-crystallized crystals was found inside a 10 Å site. This

has been accomplished by using the Protein Production Wizard. With the use of the OPLS2005 forcefield and the maestro's ligand preparation tool, the ligands containing the reference molecule were able to produce their possible conformers, tautomers, or stereoisomers. These synthetic ligands with energy minimization were used for molecular docking studies with the XP mode and a Van der Scaling of 0.8.

MMGB/SA calculations

In computational chemistry and structural biology, the binding free energy between proteins and ligands (small molecules) is calculated using a computer method called MMGB/SA (Molecular Mechanics/Generalized Bom Surface Area). It is widely used in drug development and protein-ligand interaction studies to forecast the binding affinity of possible therapeutic candidates to target proteins. The MMGBSA approach combines surface area calculations, molecular mechanics, which uses force fields to characterise a system's internal energy and generalised Born solvent models. The binding free energy, or G_bind, is estimated as follows:

$$\Delta G\_bind = \Delta E\_MM + \Delta G\_solv - T\Delta S$$

In molecular mechanics, E MM refers to the energy change that happens after binding. This energy change is determined using the GB model and SA calculations, which yield the solvation-free energy change, or G solv. Generally, in MMGBSA simulations, the change in entropy upon binding, or TS, is either ignored or estimated.

ADMET Prediction

ProTox-II was used for toxicity prediction. For the best scores obtained from the MM-GBSA research, QikProp v6.8 and SwissADME were utilised to calculate ADME characteristics. The result consists of several ADMET property predictions.

Formulation of cream

The cream's formulation was created in two stages: the aqueous phase contained distilled water, glycerine and propylene glycol, while the oil phase contained stearic acid and cetyl alcohol. To create a homogenous cream mixture, the oil and aqueous phases were melted at 70°C while being stirred with a homogenizer. In addition, sodium benzoate was added to the mixture to improve stability and shelf life. Upon cooling, the cream base was mixed with ferulic acid and chlorogenic acid.[19,20] The same process was used for developing three formulations, designated F1, F2 and F3, with different ratios of ferulic acid to chlorogenic acid. Table 1 displays the developed cream formula.

Physical evaluation of cream

Organoleptic properties

This includes the color, consistency and appearance of the cream. A cream should ideally have a smooth, homogeneous look and a color that complements its constituents. The odour of the cream should be pleasant and consistent with the components. The texture of the cream on hands should be spreadable, non-greasy and smooth.[21]

Spreadability

A parallel plate method was used to assess spreadability. An excess of the sample was sandwiched between two slides and compressed using a specific weight for a certain period of time to achieve a consistent thickness. Next, spreadability was assessed by measuring the duration of time needed to separate the slides. Better spreadability properties are thus correlated with shorter separation periods.[22]

Determination of pH

The pH of the prepared cream was measured accurately in order to assess its compatibility with healthy skin (pH range: 5-6). The 5.00±0.01 g of cream was weighed accurately and transferred to a 100 mL beaker. 45 mL of distilled water were added to the cream in order to promote uniform dispersion. After that, the pH of the resulting solution was measured at a controlled temperature of 27°C using a calibrated pH metre.

Stability studies of formulation

Stability testing of pharmaceutical products is a crucial step in the development process that begins with the discovery phase and continues until product discontinuation. Following ICH specifications in this investigation ensured a consistent procedure for evaluating the stability of the drug substances and their formulated creams. The cream was sealed into bottles and kept in specially designed chambers with regulated humidity levels. 30°C±2°C and 65%±5% Relative Humidity (RH) and 40°C±2°C and 75%±5% RH were the two storage conditions used. Cream samples were subjected to assessments after a 15 days storage period in order to assess variations in their viscosity and physical characteristics.[23]

Pharmacological activity

In vitro protein denaturation assay for anti-inflammatory activity

Egg albumin solutions were prepared with a total volume of 10 mL in concentrations of 50 and 100 µg/mL. 0.4 mL of fresh chicken egg albumin, 5.6 mL of phosphate buffer solution with a pH of 6.4 and 4 mL of cream test sample were added to the mixture. The same volume of DMSO was used as a control. All mixes were preheated for 5 min at 70°C and then they were incubated for 15 min at 37°C±2°C. UV spectrophotometer was used to detect

the absorbance at 280 nm after incubation and cooling, with vehicle solution serving as a blank. Using the same procedure, the final concentrations of 50 and 100 µg/mL of indomethacin were measured as a reference.[24]

In vitro DPPH assay for antioxidant activity

DPPH 0.1 mM solution was prepared in methanol along with various quantities (25, 50, 75 and 100 µg/mL) of the test sample. The standard was 100 µg/mL of ascorbic acid, while the control was 1 mL of methanol and 2 mL of DPPH solution. Using methanol as the blank, absorbance was measured at 516 nm using a spectrophotometer after a combination of 2 mL of the test sample at various concentrations and 2 mL of DPPH solution was incubated for 10 min in darkness. With the use of the given formula, the percentage inhibition of the DPPH radical was estimated.[25]

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

A_{sample} denotes the absorbance of the test sample, whereas $A_{control}$ denotes the absorbance of the control reaction, which comprises all reagents save the test sample. Plotting the percentage inhibition versus the sample concentration allowed for the determination of the IC_{50} value.

RESULTS

Molecular docking studies

Computational analysis was utilised to further validate the hypothesis by utilising the compounds with well-predicted IC_{50} values, as shown in Table 2. Molecular docking is used to anticipate the interactions between molecules, such as small new HITs or ligands and a target macromolecule, often a protein. It involves estimating the ligand-protein binding mechanism and affinity.

Protein preparation and receptor grid generation for anti-inflammatory activity: In conjunction with indomethacin 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid, the crystal structure of the PPAR-γ ligand Binding Domain (PDB ID: 4XUM) was acquired. A grid around the protein's active site was generated using Glide v9.1's receptor grid creation panel after the protein structure had been processed using the protein preparation wizard panel. The protein structure is suitable for further computational analysis and docking.

Protein preparation and receptor grid generation for anti-oxidant activity

The Glutathione S-transferase omega-1 complex with ascorbic acid crystal structure (PDB ID: 3VLN) was obtained from the Protein Data Bank. After processing the protein structure using the protein preparation wizard panel, a receptor grid creation panel in Glide v9.1 was utilised to generate a grid encircling the active sites of the protein. The rigorous processing confirms that the protein structure is suitable for further computing and docking studies.

Ligand Preparation

The 2D sketcher was used to first depict the compounds and then, using the OPLS 5 force field, geometric minimization was used to optimise them. In order to assure that compound structures were refined for further investigation, Ligprep was used. This process is crucial for improving computational study accuracy and for producing a more trustworthy depiction of the molecular characteristics and interactions inside the compounds.

Table 1: Composition and Evaluation of Herbal Cream Formulations Containing Chlorogenic Acid and Ferulic Acid.

Composition of Cream Formulations			
Ingredients	**F[1]1**	**F[1]2**	**F[1]3**
FA[3]: CA[2]	1.20 g: 30 mg	0.60 mg: 30 mg	0.30 mg: 0.30 mg
Stearic acid	6 g	6 g	6 g
Cetyl alcohol	3 g	3 g	3 g
Propylene glycol	14 g	14 g	14 g
Glycerine	2.5 mL	2.5 mL	2.5 mL
Sodium benzoate	0.025 g	0.025 g	0.025 g
Distilled water	q.s.	q.s.	q.s.
Evaluation of Cream Formulations			
Test Parameter	**Test Formulation**	**Std[4] Formulation**	
Colour	Off-white	Off-white	
Odour	Characteristic	Characteristic	
Appearance	Semi-solid	Semi-solid	
Spreadability	7.1 g·cm/sec	7.3 g·cm/sec	

F[1]=Formulation, CA[2]=Chlorogenic acid, FA[3]=Ferulic acid, Std[4]=Standard marketed formulation.

Table 2: Molecular Docking Scores, Binding Energies, and Interactions of Test Compounds with PPAR-γ and GST Omega-1 for Anti-inflammatory and Antioxidant Activities.

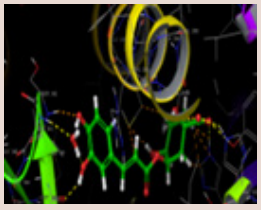
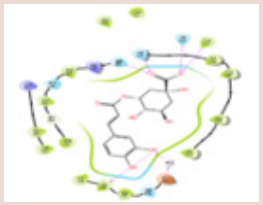
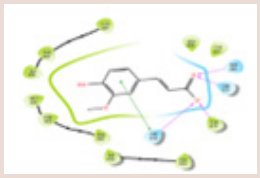
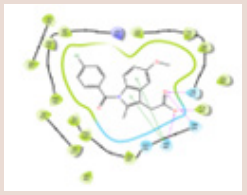
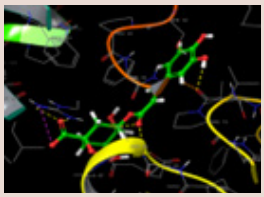
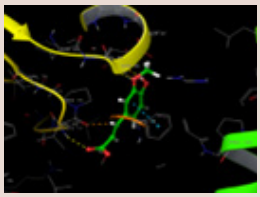
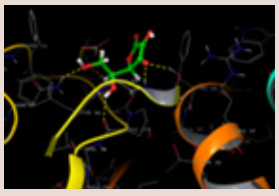
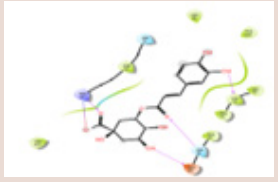
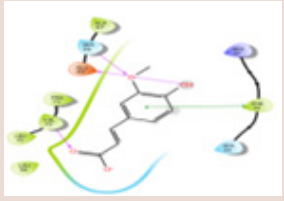
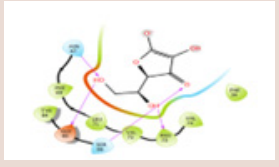
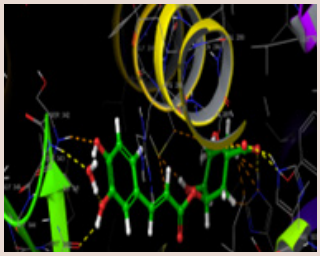
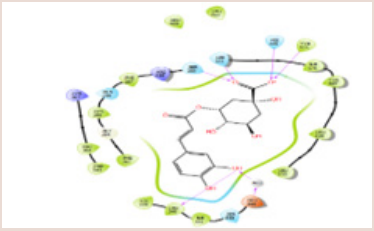
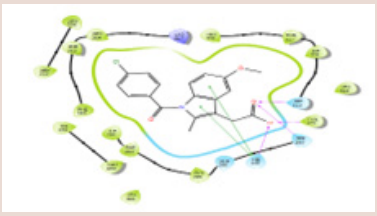
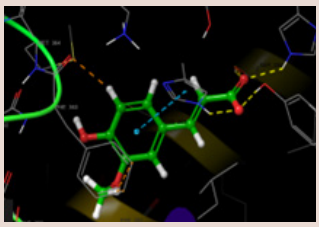
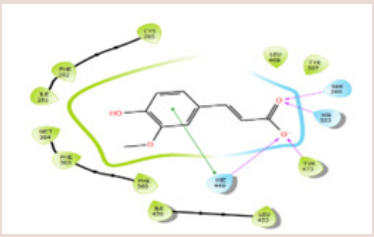
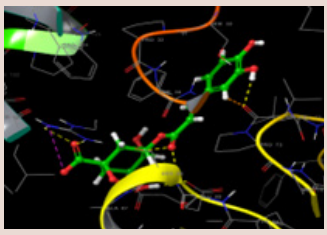
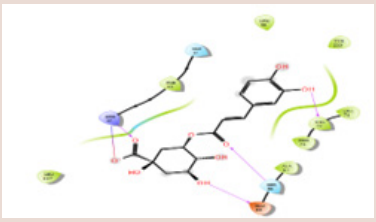
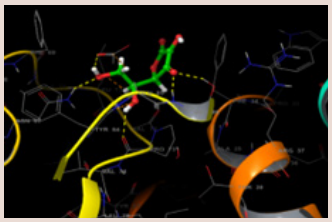
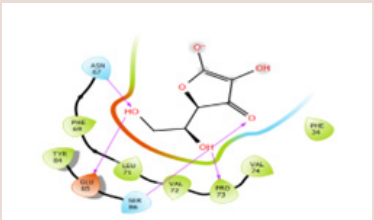
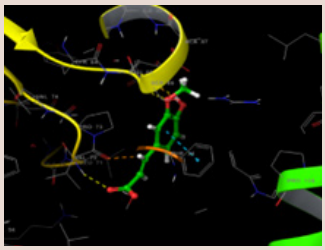
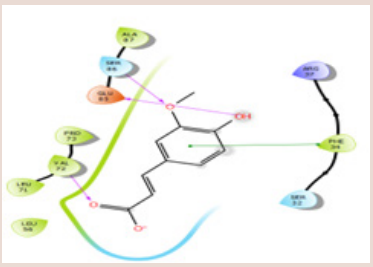
Parameter	Chlorogenic Acid	Ferulic Acid	Indomethacin	Ascorbic Acid
Dock Score (PPAR-γ)	-13.236	-7.313	-13.109	-
dG Binding (PPAR-γ)	-65.69	-44.93	-101.09	-
Dock Score (GST omega-1)	-7.829	-4.758	-	-7.164
dG Binding (GST omega-1)	-58.48	-25.95	-	-30.71
2D Interactions (GST omega-1)	H-bond: ARG37, GLU85, VAL72, SER86 Salt-Bridge: ARG37	H-bond: GLU85, SER86, VAL72 Pi-Pi Stacking: PHE34	-	H-bond: ASN67, GLU85, SER86, PRO73
Distance (GST omega-1) (Å)	1.59, 1.80, 2.23, 1.86 4.85	1.60, 2.10, 2.19 4.07	-	2.04, 1.81, 1.83, 2.05
2D Interactions (PPAR-γ)	H-bond: SER289, HIE323, HIE449, TYR473, LEU340, GLU343, H2O	H-bond: SER289, HIE323, HIE449, TYR473 Pi-Pi Stacking: HIE449	Pi-Pi Stacking: HIE449, HIE449 H-bond: SER289, HIE323, TYR473, HIE449	-
Distance (PPAR-γ) (Å)	1.66, 2.34, 1.85, 1.86, 1.98, 2.24, 2.19	1.70, 2.21, 1.95, 1.75	4.60, 4.74 1.88, 1.96, 1.75, 1.99	-
2D & 3D Interaction Images (PPAR-γ)				-
				-
2D & 3D Interaction Images (GST omega-1)			-	
			-	

Table 3: 2D and 3D interaction images of chlorogenic acid, ferulic acid, and reference compounds.

Interaction images of all molecules with Peroxisome proliferator-activated receptor gamma (PPARgamma) (PDB ID: 4XUM):		
Compound Name	**3D Image**	**2D Image**
Chlorogenic acid		
Reference Indomethacin		
Ferulic acid		
Interaction images of all molecules with Glutathione S-transferase omega-1 Complex with Ascorbic Acid (PDB ID: 3VLN):		
Chlorogenic acid		
Reference Ascorbic acid		
Ferulic acid		

Ligand Docking with Glide

Open the Glide application, then select the docking technique between Standard Precision (SP) and Extra Precision (XP). Even though XP requires more computing resources, it provides more precision. Set up the ligand flexibility, position sampling, scoring functions and docking parameters. Table 2 displays the molecular docking scores.

MM-GBSA

The process of Prime/MM-GBSA was utilised to ascertain the relative binding energy of selected ligands. The XP docking-derived pv.maegz file was used as the study's input for the MM-GBSA. As shown in Table 2, the protein's active site was designed to adjust within a range of 5 o' A from the ligand. This approach assures a thorough investigation of ligand-protein interactions, accounting for the active site's flexibility and offering insightful information on the energetics of ligand binding. It is more likely that the compounds with lower (more negative) total binding free energies will interact significantly with the target protein, making them excellent candidates for more research. Binding interactions of all molecules with the receptors Glutathione S-transferase omega-1 Complex for antioxidant activity and PPAR-γ for antioxidant activity are shown in Table 2. Interaction images of all molecules with Glutathione S-transferase omega-1 Complex and PPAR-γ are shown in Table 3.

ADMET Prediction

To determine ADME properties, QikProp v6.8 and SwissADME were employed. QikProp was used to evaluate the compounds' different ADME (Absorption, Distribution, Metabolism and Excretion) properties. These attributes encompass a wide range of crucial factors, offering an extensive understanding of the pharmacokinetic and drug-like properties. The evaluated features include Central Nervous System interaction, Solvent Accessible Surface Area, Polar Surface Area (PSA), Madin Darby Canine Kidney Cell Line Permeability (MDCK), Number of Nitrogen and Oxygen atoms (NandO), log hERG (Human ether-à-go-go-related gene), predicted aqueous solubility (LogS), Acceptor Hydrogen Bond (Accept HB), predicted octanol/water partition coefficient (Log Po/w), predicted blood/brain partition coefficient (Log BB), prediction of binding to human serum albumin (Log Khsa), Donor Hydrogen Bonds (Donor HB), predicted qualitative human oral absorption (HOA, with values ranging from 1 for low, 2 for medium and 3 for high), number of Rule of Five violations and Caco-2 permeability (PCaco). These thorough analyses provide detailed data on the pharmacological potential of the compounds, which helps determine whether or not they are suitable for drug development. The ADME study of molecules by QikProp v6.8 is shown in Table 4.

The compounds' varied ADME (Absorption, Distribution, Metabolism and Excretion) characteristics were evaluated using SwissADME. These attributes encompass a wide range of crucial factors, offering a comprehensive comprehension of the pharmacokinetic and drug-like characteristics. Molecular weight, the number of Rule of Five violations, GI Absorption, Bioavailability Score, Synthetic accessibility and the predicted octanol/water partition coefficient (Log Po/w) and blood/brain partition coefficient (Log BB) are among the evaluated features. These thorough analyses provide insightful information on the pharmacological potential of the compounds, which helps determine whether or not they are suitable for drug development. The ADME study of molecules by SwissADME is shown in Table 4.

ProTox-II was utilised to forecast toxicity for the optimal score derived from the MM-GBSA study and Docking Score. The molecules were also exposed to toxicity prediction for hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity and immunotoxicity using the ProTox- II portal, as shown in Table 4.

Physical evaluation of cream

With a pH of around 5.7, the developed cream showed good compatibility with healthy skin, which normally has a pH range of 5 to 6. To evaluate the cream's colour, odour, spreadability and overall appearance, an organoleptic evaluation was performed, as shown in Table 5. The developed creams showed not much variation in terms of appearance, pH, odour and phase separation during a 15-day stability study period. In addition, no phase separation was seen during this duration. Table 5 includes a summary of the stability study results.

Pharmacologica Activity

In vitro anti-inflammatory study: A protein denaturation experiment was carried out *in vitro* to evaluate the anti-inflammatory characteristics of different formulations. Formulation F1 is more effective than the others in terms of protein denaturation inhibition, exhibiting a dose-dependent response. Inhibition was maximum in the test sample at 50 µg/mL (70.31 µg/mL % MIC) and 100 µg/mL (74.11 µg/mL % MIC) concentrations. whereas the reference anti-inflammatory drug indomethacin had a % MIC of 75.39 µg/mL (Table 5).

In vitro antioxidant study

A DPPH assay was carried out to assess the antioxidant properties of ferulic and chlorogenic acids, which are known for their capacity to scavenge free radicals. The sample changed colour from a deep purple to a pale yellow due to the presence of these antioxidants. Formulated cream showed maximum inhibition at 75 µg/mL and 100 µg/mL with 83.89 and 90.67% inhibition. This assay demonstrates efficient antioxidant activity for cream when compared to ascorbic acid, which shows the highest inhibition (Table 5).

Table 4: Comprehensive ADME and Toxicity Profile of Chlorogenic Acid, Ferulic Acid, Indomethacin, and Ascorbic Acid.

Parameter	Chlorogenic Acid	Ferulic Acid	Indomethacin	Ascorbic Acid
ADME study of molecules by QikProp v6.8.				
CNS	-2	-2	-1	-
SASA	607.518	407.334	598.586	-
Donor HB	6.000	2.000	1.000	-
Acceptor HB	9.650	3.500	5.750	-
Log Po/w	-0.268	1.400	4.261	-
Log S	-2.529	-1.701	-5.130	-
Log HERG	-3.290	-2.049	-3.364	-
PCaco	1.696	81.204	181.654	-
Log BB	-3.340	-1.041	-0.641	-
PMDCK	0.637	41.703	245.547	-
Log Khsa	-0.926	-0.629	0.052	-
HOA	1	3	3	-
PSA	184.193	80.435	82.301	-
N and O	9	4	5	-
Rule of Five	1	0	0	-
ADME study of molecules by SwissADME				
MW	354.31 g/mol	194.18 g/mol	-	176.12 g/mol
H-bond Acceptor	9	4	-	6
H-bond Donors	6	2	-	4
Log P	0.96	1.62	-	0.39
GI Absorption	Low	High	-	High
BBB Permeant	No	Yes	-	No
Bioavailability Score	0.11	0.85	-	0.56
Lipinski Violation	1	0	-	0
Synthetic Accessibility	4.16	1.93	-	3.47
Toxicity study of molecules by ProTox-II.				
Hepatotoxicity	Inactive	Less Inactive	Active	Inactive
Carcinogenicity	Less Inactive	Less Inactive	Less Active	Inactive
Immunotoxicity	Active	Active	Less Active	Inactive
Mutagenicity	Inactive	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Less Inactive
ProTox-II Class	5	4	2	5

DISCUSSION

A molecular docking study reveals that chlorogenic acid and ferulic acid showed excellent antioxidant and anti-inflammatory effects compared to ascorbic acid and indomethacin. Docking studies showed exceptional binding affinity towards the well-characterized crystal structures of Glutathione S-transferase omega-1 complex and PPAR-γ by chlorogenic acid and ferulic acid. By using MM-GBSA binding free energies calculated, a higher negative value indicates more favourable interactions between the ligands and target proteins. The ADMET study of all compounds reveals that chlorogenic acid and ferulic acid come in classes 5 and 4, respectively, while indomethacin and ascorbic acid come in classes 2 and 5, respectively. Hepatotoxicity, cytotoxicity, carcinogenicity and mutagenicity were absent from both drugs. Both compounds don't have any severe side effects, which indicates their suitability for pharmaceutical applications. The formulation of both compounds demonstrated compliance with all physical parameters and stability parameters, showing a pH 5.7, which complies with skin pH. The formulated cream exhibited excellent *in vitro* efficacy for both anti-inflammatory

Table 5: Stability, Protein Denaturation, and DPPH Assay Results for the Formulated Cream.

Section	Parameter	Condition / Concentration	Result
Stability Studies	Physical Appearance	30±2°C / 65 ± 5% RH[1]-Initial	Semi-solid
		30±2°C / 65 ± 5% RH[1]-After 15 Days	Semi-solid
		40±2°C / 75±5% RH[1]-Initial	Semi-solid
		40±2°C / 75±5% RH-After 15 Days	Semi-solid
	Texture	All Conditions	Fine
	Colour	All Conditions	Off-white
	Odour	All Conditions	Characteristic
	pH	30±2°C-Initial / After 15 Days	5.7 /5.8
		40±2°C-Initial / After 15 Days	5.6 / 5.7
Anti-inflammatory Activity	Egg Albumin Assay	25 µg/mL	57.18
		50 µg/mL	70.31
		100 µg/mL	74.11
		Reference Standard (Indomethacin)	75.39% Inhibition
Antioxidant Activity	DPPH Assay	25 µg/mL	30.50% Inhibition
		50 µg/mL	33.05% Inhibition
		75 µg/mL	49.15% Inhibition
		100 µg/mL	83.89% Inhibition
		Reference Standard (Ascorbic Acid)	90.67% Inhibition

[1]RH=Relative humidity.

and antioxidant activities. *In vivo* research might be conducted to acquire a further understanding of these creams' effectiveness.

CONCLUSION

The docking study reveals chlorogenic acid and ferulic acid have strong binding affinity to target proteins, which exhibit antioxidant and anti-inflammatory activities when compared to their reference standards and ADMET studies of chlorogenic acid and ferulic acid showed their effectiveness in the application of pharmaceutical products. The formulated cream showed excellent stability and met physical parameters, showing a pH of 5.7, which indicates its suitability for external use on skin. *In vitro* studies of formulated creams for antioxidant and anti-inflammatory activities gave promising results. However, to comprehensively evaluate the efficacy of the cream's therapeutic potential, further *in vivo* investigations are required.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ADMET: Adsorption Degradation Metabolism, Excretion, Toxicity; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **NR1C3:** Nuclear Receptor Subfamily 1 Group C Member 3; **GSTO1:** Glutathione S-transferase Omega-1; **OPLS2005:** Optimized Potentials For Liquid Simulations 2005; **XP:** Extra Precision; **MM-GBSA:** Molecular Mechanics Generalized Born Surface Area; **ICH:** International Council For Harmonization; **DMSO:** Dimethyl Sulfoxide.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Human participants or animal experiments were not used in this study. Since this study did not include human participants, informed consent was not applicable.

SUMMARY

This work provides a comprehensive overview of ADMET prediction, physical evaluation of formulated cream, pharmacological activity and molecular docking studies of ferulic acid and chlorogenic acid performed with Schrödinger software. This study investigates the ADMET properties of the compounds, providing information on their pharmacokinetic and drug-like characteristics through detailed evaluations utilising QikProp v6.8, ProTox-II and SwissADME software. the formulated cream demonstrated stability and compatibility

over the duration of stability period. promising outcomes were obtained from pharmacological studies. Specifically, Formulation F1 demonstrated a significant dose-dependent inhibition of protein denaturation in an *in vitro* anti-inflammatory studies. Furthermore, the cream exhibited strong antioxidant activity in an *in vitro* study. The binding interactions between the compounds and their individual molecular targets were further explained, revealing on the potential mechanisms behind the compounds' pharmacological effects, through the use of Schrödinger software in molecular docking simulations. This research offers an integrated approach to investigate the cream's therapeutic potential as an effective topical treatment for oxidative stress reduction and inflammation control.

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