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Mechanistic Insight Diuretic activity of *Tacoma stans* leaf Bioactive Flavonoid against *UT-1* Protein: Molecular Docking Validation

Chhaya Rathor[1*], D K Sharma[1], Shobharam Sahu[2]

[1*]School of Pharmacy and Research Centre, Sanskriti University, 28 K. M. Stone, Mathura - Delhi Highway, Chhata, Mathura UP, 281401

[2]Rajiv Gandhi College of Pharmacy, By Pass Road, Nautanwa, Maharajganj UP. 273164

Corresponding Author Email:chhaya05ntv@gmail.com

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ABSTRACT

Background: Botanicals have long been an important part of the treatment of chronic and infected wounds, even if modern science still has a limited knowledge of the molecular basis of these medications. Diuretics are drugs that increase the flow of urine and salt excretion. They are employed in a variety of therapeutic conditions to change the volume and content of body fluids. In clinical practice, most complete diuretics are used to reduce extracellular fluid volume by lowering total body NaCl levels. Electrolyte imbalance and metabolic alterations are common adverse effects of today's diuretics, such as thiazides and loop diuretics. *Tecoma stans* (L.) Juss. ex Kunth (Bignoniaceae) is an attractive evergreen plant known as kusi urakame, koyawari, Palo amarillo, tronadora, yellow-elder, yellow trumpet bush, trumpet-flower, yellow-bells, trumpet bush, ginger-Thomas, esperanza, and timboco. It is widely used in traditional Mexican medicine, to treat hyperglycemia, gastrointestinal and urinary tract disorders, jaundice, toothaches, headaches, colds, skin infections, and scorpion, snake, and rat bites. Current research focusses on evaluating its bioactive components and therapeutic potential. Tecoma stans confirmed its origin, ethnopharmacological and therapeutic uses.

Method: In the current study, a molecular docking technique was used to try and identify ***UT-1*** protein inhibitors. A grid-based docking strategy was used to determine the binding using the Auto Dock software.

Result:*TS leaf* found to be effective diuretic agent and their lead molecules effectively binds to be target protein ***UT-1*** enzyme with binding energy -3.06 & -3.54 kcalmol^{-1} for chlorogenic acid & caffeic acid respectively.

Conclusion: A computationally based docking investigation revealed that both lead compound (chlorogenic acid and caffeic acid) has potent ***UT-1*** inhibitory properties.

Key words: Diuretic acid, chlorogenic acid, caffeic acid, *UT-1*& molecular docking.

Introduction

Diuretics are drugs that increase the flow of urine and salt excretion. They are employed in a variety of therapeutic conditions to change the volume and content of body fluids. In clinical practice, most complete diuretics are used to reduce extracellular fluid volume by lowering total body NaCl levels. Electrolyte imbalance and metabolic alterations are common adverse effects of today's diuretics, such as thiazides and loop diuretics. In recent years, medicinal plants have been considered as a rich source of therapeutic substances for disease prevention, and their health benefits have been rapidly expanding. This is probably due to the effects of some plants which are similar to those of allopathic drugs. Although, traditional treatments can help with several ailments, yet they need to be scientifically validated before they can be used to their full potential. The mode of action of the bulk of these traditional medicines has yet to be determined because there has been no regulatory authority to assess the appropriate use of traditional medicines. As a result, the efficacy of these traditional herbal treatments must be determined[1-2].

Tecoma stans (L.) Juss. ex Kunth (Bignoniaceae) is an attractive evergreen plant known as kusi urakame, koyawari, Palo amarillo, tronadora, yellow-elder, yellow trumpet bush, trumpet-flower, yellow-bells, trumpet bush, ginger-Thomas, esperanza, and timboco. It is widely used in traditional Mexican medicine, to treat hyperglycemia, gastrointestinal and urinary tract disorders, jaundice, toothaches, headaches, colds, skin infections, and scorpion, snake, and rat bites. Current research focusses on evaluating its bioactive components and therapeutic potential. Tecoma stans confirmed its origin, ethnopharmacological and therapeutic uses. More than 120 chemical compounds have been isolated, and the main active principles are alkaloids, phenolic acids, flavonoids, and fatty acids. The plant possesses vast therapeutic benefits, such as lowering elevated blood sugar levels, anti-inflammatory, anti-cancer, anti-bacterial, anti-fungal, anti-oxidant, hepatoprotective, and wound healing actions[3-4]. As per literature review various flavonoids and plant phenolic present in leaf extract therefore, chlorogenic acid and caffeic acid was chosen as lead bioactive molecule for *in-silico* molecular docking.Urine concentration is greatly impacted by urea transporters (UTs). The two subgroups of UTs are UT-A and UT-B. Six isoforms of the UT-A subfamily, UT-A1 through UT-A6, are produced via alternative splicing and promoters from a single gene, SLC14a2. Although endogenous expression of UT-A4 protein in the kidney has not been confirmed, UT-A1 and UT-A3 are expressed in the renal inner medullary collecting duct, UT-A2 is found in the thin descending limb, and UT-A4 mRNA is found in the rat kidney medulla. Both UT-A5 and UT-A6 are found in the colon and testes, respectively. The descending vasa recta, as well as the heart, colon, testis, bladder, brain, and erythrocytes, are the primary sites of UT-B, which is encoded by the gene SLC14a1. According to earlier research, selective UT deletion can reduce the kidney's capacity to concentrate urine and create a urea-selective diuretic effect without impacting the excretion of Na+, K+, and Cl. Therefore, it is believed that UT inhibitors can have a diuretic effect by preventing urea recycling in the kidney, thereby lowering the osmotic pressure gradient in the kidney [5].

Experimental works

***In-Silico* molecular docking study**

Selection of lead bioactive compound:

As per literature survey an investigation carried out by **Dhanashri Jadhav etal; 2023** leaf showed presence of many (Bioactive compounds) which are showing as follows (6) as Tecostatin, Boschiakine, Luteolin, Caffeic acid, Chlorogenic acid& Gallic acid.So, in order to assess the diuretic potential of Caffeic acid and chlorogenic acid was selected as lead compound for virtual screening and elucidate the proposed mechanism of action.

Molecular docking studies

Ligand Preparation:

2D Structure of ligands like caffeic acid and chlorogenic acid were drawn using ChemSketch [7], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

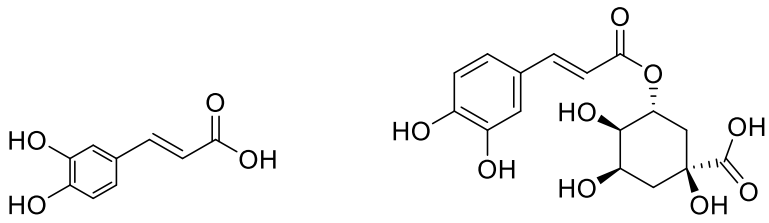


Fig 1: 2D structure of caffeic acid and chlorogenic acid.

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing and grid points is given in table 1 [8-9].

Table 1. Grid parameters used in current docking analysis of UT1.

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	UT1	40	40	54	0.408	130.419	85.092	-8.378

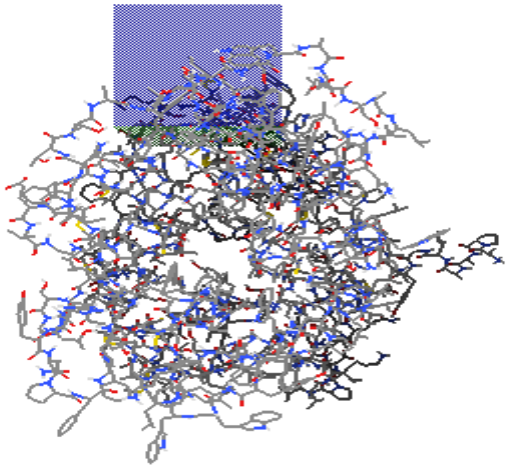


Fig 2: Grid box covering all active sites in UT1 receptor

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [9].

Crystal structure

The crystal structure of the protein consisting of UT1 receptor is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (6dq5.pdb) registered in the Protein data bank was used [10]. The complex ligand was separated by using Chimera software.

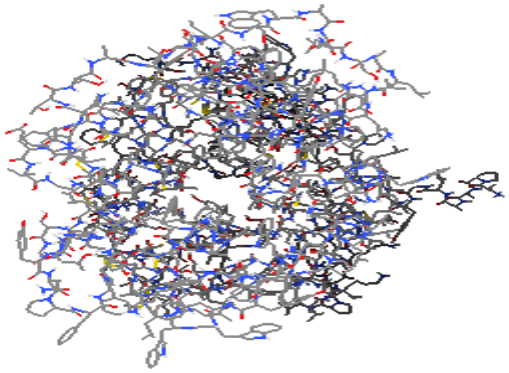


Fig 3: Crystal structure of UT1 receptor (PDB ID-6dq5)

Processing of Protein

The downloaded receptor protein is having only one chains, i.e. chain A, which has been selected for experimental purpose and complex ligand was removed from it. The bound ligand was separated from the macromolecular complex by using software Chimera [11].

Molecular Docking Simulation Studies

Docking of ligands like caffeic acid and chlorogenic acid against UT1 receptor was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [12].

Fig 4: Binding mode of chlorogenic acid within the active site of UT1 receptor.

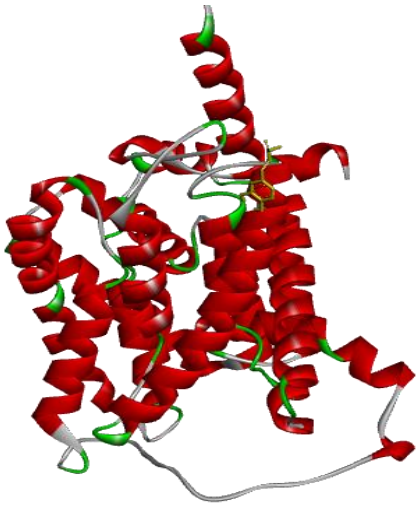


Fig 5: Binding mode of caffeic acid within the active site of UT1 receptor.

Toxicity & ADME-T Studies

The ligand molecules viz. caffeic acid and chlorogenic acid were studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [13].

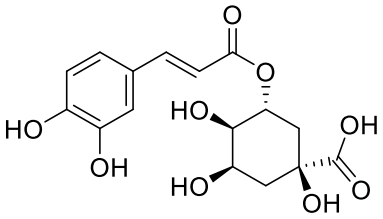
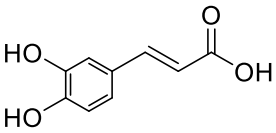
Result & Discussion

TS leaf found to be effective diuretic agent and their lead molecules effectively binds to be target protein ***UT-1*** enzyme with binding energy -3.06 & -3.54 kcalmol^{-1} for chlorogenic acid & caffeic acid respectively. The result was tabulated in table 2 & fig 6. The grid parameter used in current docking analysis of ***UT-1*** was showed in table 1& fig.1.The binding mode of selected lead molecules showed in fig.4-5. The 2D and 3D interaction of selected compound displayed in fig.7-12. The interaction of chlorogenic acid & caffeic acid with active site at ***UT-1*** enzyme showed as follows:

Compound	**Conventional Hydrogen bounding**	**Pi-sigma bounding**	**Covalent bounding**	**Week Vander's interaction**
Chlorogenic acid	Leu^{313}	-	Gln^{307}	Leu^{266},Leu^{317},Leu^{310},Thr^{308} Trp^{306},Ile^{359},Leu^{311},$Phen^{349}$ Met^{352}
Caffeic acid	-	Leu^{310}	Glu^{307}	Asn^{358},Asn^{356},Ile^{359},Thr^{308} Leu^{311},Try^{306}

The pharmacokinetic profile reveals that it is having good pharmacokinetic profile but with the presence of any major toxic effects including mutagenicity, tumorogenicity and reproductive effects. The pharmacokinetic and toxicity profiling results of ligands like caffeic acid & chlorogenic acid were shown in figure 13-14. Theoretically, all the ligand molecules have shown encouraging docking score.

Table 2: Results of docking of ligands like caffeic acid and chlorogenic acid against UT1 receptor.

Sl. No	CompoundName	Structure	BindingEne
1	**Chlorogenic acid**		-3.06
2	**Caffeic acid**		-3.54

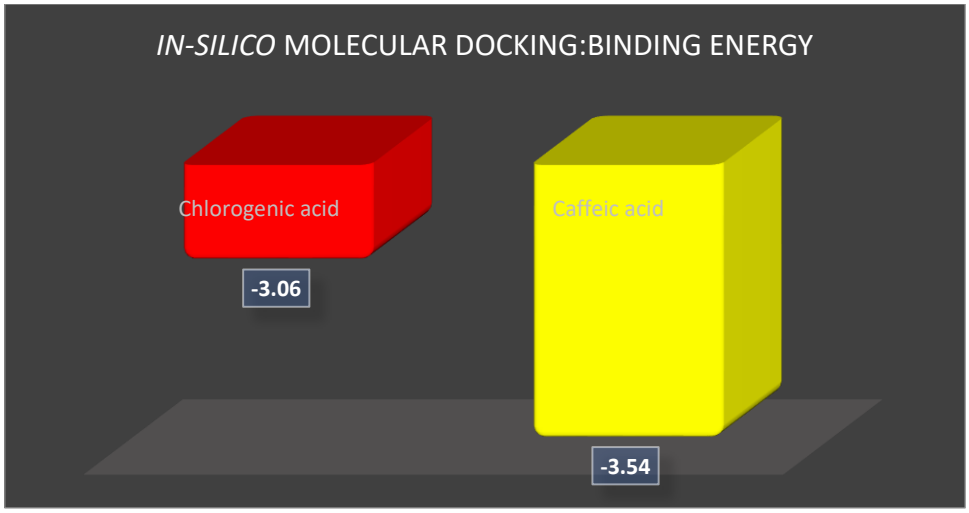


Fig 6: *In-Silico* Molecular Docking: Binding Energies

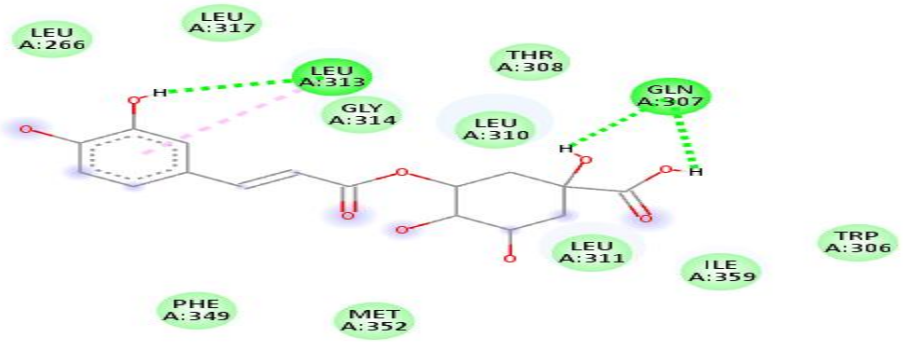


Fig 7: Two-dimensional binding mode of chlorogenic acid within the active site of UT1 receptor

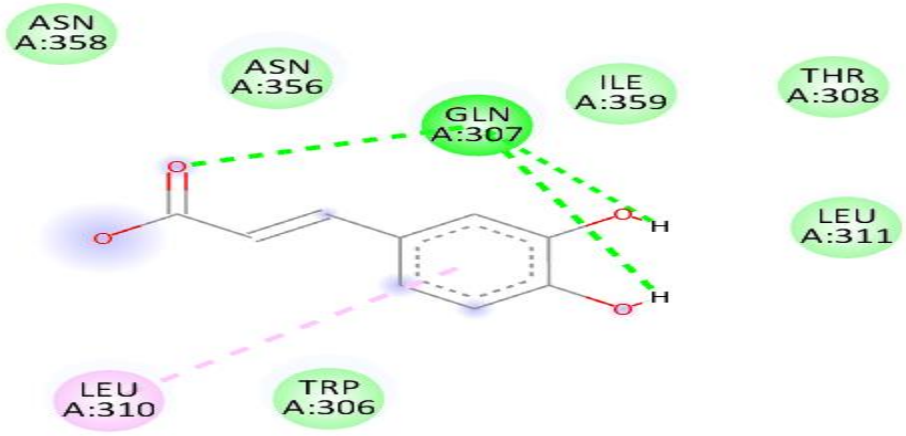


Fig 8: Two-dimensional binding mode of caffeic acid within the active site of UT1 receptor

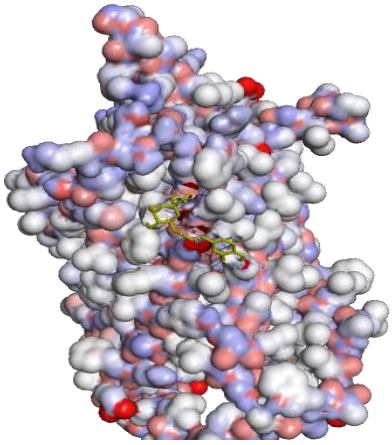


Fig 9: Three-dimensional binding conformation of chlorogenic acid within the active site of UT1 receptor

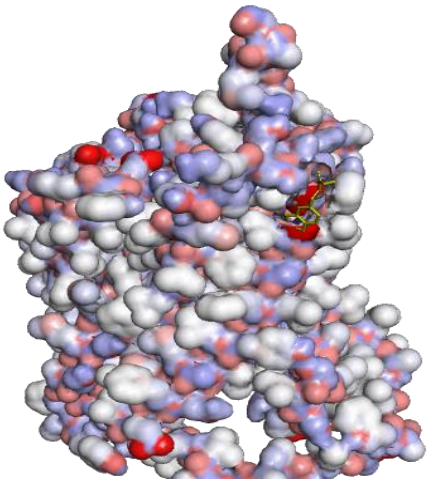


Fig 10: Three-dimensional binding conformation of caffeic acid within the active site of UT1 receptor

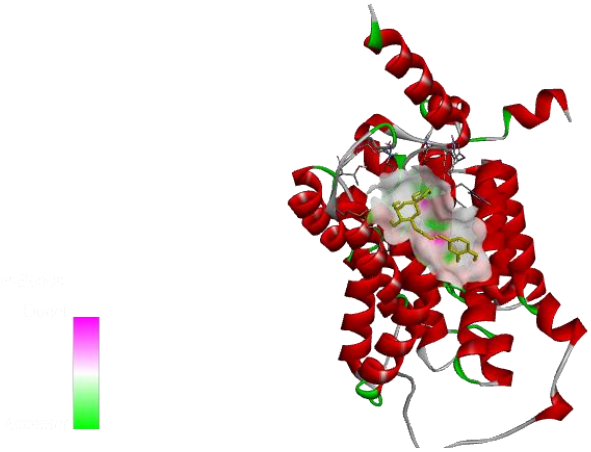


Fig 11: Three-dimensional binding mode of chlorogenic acid within the active site of UT1 receptor

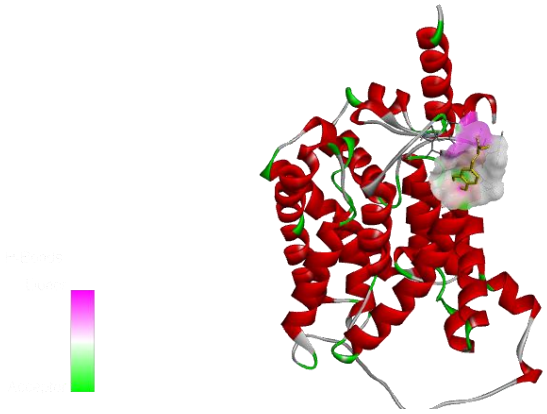


Fig12: Three-dimensional binding mode of caffeic acid within the active site of UT1 receptor

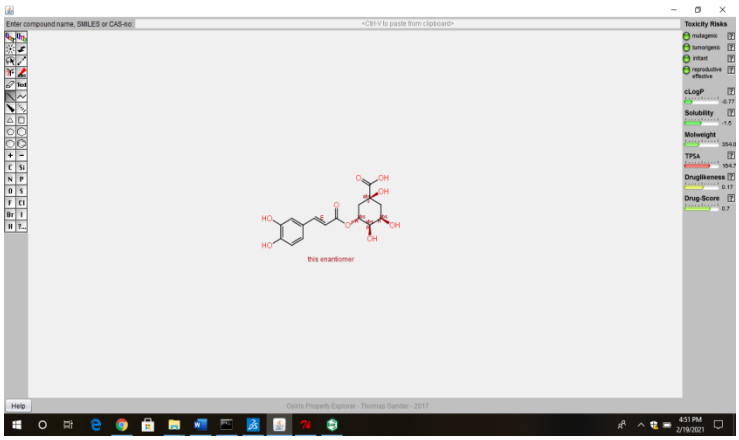


Fig 13: Pharmacokinetic and toxicity profiling of chlorogenic acid.

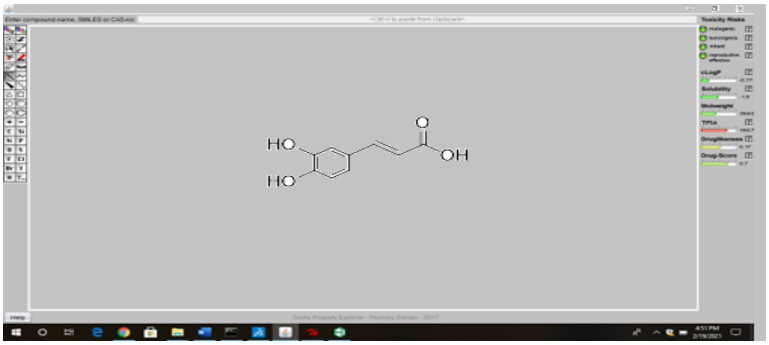
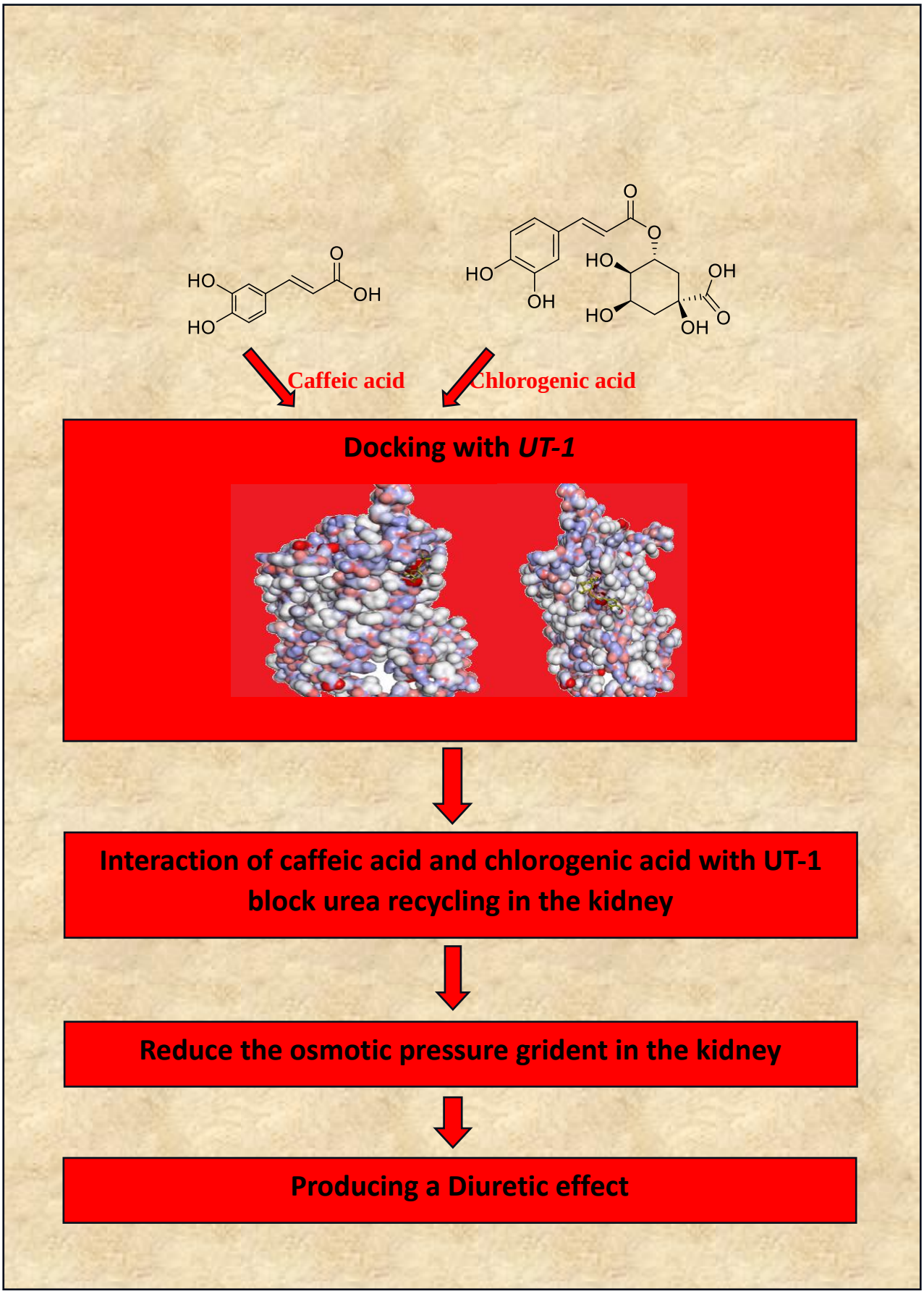


Fig 14: Pharmacokinetic and toxicity profiling of caffeic acid.

Divulgence of Investigation

The in-silico molecular modelling investigation's findings demonstrated that the aqueous leaf *Tecoma stans* extract's showed significant diuretic efficacy was due to the presence of caffeic acid and chlorogenic acid. The suggested mechanism is illustrated as follows:


HO
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Caffeic acid
Chlorogenic acid
Docking with *UT-1*
Interaction of caffeic acid and chlorogenic acid with UT-1 block urea recycling in the kidney
Reduce the osmotic pressure grident in the kidney
Producing a Diuretic effect

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