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Unveiling geniposide from *Paederia foetida* as a potential antihypertensive treatment: an integrated approach involving in vivo and computational methods

Chanchal Koley[1†], Arijit Mondal[2†], Suddhsattya Dey[2*], Ravi Rawat[3], Anjan Mondal[2], Naresh Kumar Rangra[4], Deeparani Urolagin[5], Padmacharan Behera[1], Dibya Lochan Mohanty[6], Ameeduzzafar Zafar[7] and Volkan Eyupoglu[8]

Abstract

Background Hypertension is one of the burning topics in today's world. Natural product can open a new window in the treatment as they are lesser side effect compared to synthetic compounds. *Paederia foetida* a naturally occurring plant has proven its biological importance in many aspects. In this present study, the ethanolic extract of *Paederia foetida* has effectively proven its antihypertensive activity against Amphetamine-induced hypertension.

Results The study was carried out for 4 weeks with five different groups where the groups receiving *Paederia foetida* (400 mg/kg) for 4 weeks result in decrease in blood pressure and was found helpful in maintaining the sodium and potassium balance in rat's serum. Amphetamine induces decreasing sodium level that can be countered by *Paederia foetida* whole plant extract. Geniposide, an active ingredient present in this plant, is having antihypertensive activity, so it was docked against different PDB IDs (3OLL, 3OLS, 5DX3, 5DXE & 6PIT), to find its anti-hypertension effectiveness through computational chemistry. The docking investigations identified that estrogen receptor (PDB ID: 3OLS) exhibited the highest possibility to be the site of action. Docking score of Geniposide with 3OLS was -8.91 which is quit comparable with the internal ligand Estradiol.

Conclusion To assess the binding affinity of Geniposide with the estrogen receptor, an additional molecular dynamics simulation was conducted. The result strongly suggests that Geniposide has the potential to function as an activator of estrogen receptor through of β-ligand binding. This key finding reveals that Geniposide may serve as a potential in the treatment of hypertension by modulating the activity of the estrogen receptor.

Keywords Hypertension, Amphetamine, *Paederia foetida*, Molecular docking, Molecular dynamics

[†]Chanchal Koley and Arijit Mondal are contributed equally to this study.

*Correspondence:
Suddhsattya Dey
kuntal.kuni@gmail.com
Full list of author information is available at the end of the article

Background

A chronic medical condition known as hypertension causes raised blood pressure in the arteries, also known as arterial hypertension. Above 140/90 mmHg of systolic and diastolic blood pressure is often considered as hypertension. Since high blood pressure is one of the major risk factors for coronary artery disease and other consequences, including heart failure, stroke, kidney disease, and diabetes, it is one of the main causes of mortality, globally [1]. According to the Global Burden of Disease study, hypertension is also regarded as the main risk factor for disability-adjusted life years, globally [2]. According to the World Health Organization, by 2025, 1.5 billion people will have hypertension, and the condition is projected to be a factor for more than 7 million fatalities annually [3]. Numerous research are focused on development of novel antihypertensive medicines and new therapy options because of the massive prevalence of hypertension, worldwide [4, 5]. There are different classes of antihypertensive medications, including angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, and calcium channel blockers used in management of blood pressure, through the control of cardiac output, peripheral vascular resistance, and circulating blood volume [6, 7]. These antihypertensive medications are widely used to treat hypertension and associated cardiovascular disorders; however, these drugs also have undesirable side effects [6]. On account of the fact that natural herbal products made from medicinal plants are thought to have lesser adverse effects, thereby can be an alternative treatments and can be preferred over the existing medication [5]. Recently, it has been revealed that medicinal herbs can reduce hypertension and can be used empirically as antihypertensive medicines [8, 9]. Oxidative stress can be considered as an early warning sign for both hypertension and cardiovascular disorders so plants as an natural antioxidant can have the capability to enhance their antihypertensive effects [10, 11]. Diuretics have a significant role in the clinical management of hypertension. These medications influence the renal tubular segments' salt re-absorption to boost kidney urine production [12, 13]. NCC (Sodium chloride co-transporters) and NKCC2 (Na–K-2Cl co-transporter) are the main targets of diuretics. Recent research has demonstrated that the NKCC2-oxidative stress-responsive kinase 1 (OSR1)-with-no-lysine kinase (WNK) signaling pathway is crucial for controlling blood pressure [14]. WNK may control sodium chloride co-transporters (NCC) through the intermediate kinases OSR1 [15, 16]. A Rubiaceae family member *Paederia foetida*, also known as Prasariniin in Sanskrit, is a climbing plant with a strong foetid odor. Its Hindi name is Gandhaprasarini, and its English name is Chinese Flower [17]. Numerous scientific studies have been conducted to examine the activity of *Paederia foetida* in the management of rheumatic diseases, anti-inflammatory, hepatoprotective, and chemolithotripsy encourages sexual energy, boosts physical strength, semen production, and creates a young glow [18–25]. *Paederia foetida* possesses paederolone, paederone, sitosterol, paederoside, and asperuloside, along with their glycosides it has been identified through phytochemical studies [26–28]. The leaves of the plant are a good source of vitamin C, alkaloids, ketone alcohol, and beta-carotene [29]. Important constituents such asasperuloside, beta-sitosterol, and lupeol are well quantified in leaves, too [30]. Asperuloside, scandoside, and paederoside are iridoid glucosides that are present in the plant's aerial portions [31]. Friedelin, campesterol, ursolic acid, hentriacontane, hentriacontanol, ceryl alcohol, palmitic acid, and methyl mercaptan ellagic acids are other components of *P. foetida*. [32]. Another Iridoid glycosides are also found in roots of this plant known as Geniposide [33]. Our focus of this study is on Geniposide.

Yang Fu et al. [14] demonstrated the ability of Geniposide to increase urine production and the excretion of salt and chloride ions. They further revealed that the antihypertensive effects of Geniposide were considered due to their inhibition of the activation of the WNK signaling pathway, which is mediated by the estrogen receptor. So, considering this key finding we performed molecular docking and molecular dynamics (MD) simulations studies of Geniposide and various PDB IDs based on the structure of estrogen receptor.

Molecular docking, a computer-based method, can be employed to predict the non-covalent interactions between a macromolecule and a small molecule [34]. Based on the scoring method molecular docking analyses, the energy and binding strength of the complexes are observed [35]. This technique is frequently employed to conduct preliminary screening of small compounds by analyzing their binding characteristics to drug target. This creates the necessary foundation for rational drug design of novel drugs that can have higher specificity and greater efficacy [36]. Molecular dynamics (MD) simulations rely on general physics model guiding inter-atomic interactions and predict the motion of each molecule in a molecular system. Molecular dynamic simulations can determine the stability and dynamic properties of these protein–ligand complexes by analyzing the statistical parameters of these simulations [37, 38].

The present study evaluates the antihypertensive activity of the ethanolic extract of *Paederia foetida* against Amphetamine-induced hypertension in Wistar rats. Subsequently, an *in silico* molecular docking and dynamic simulation studies were conducted using the key phytochemical constituent, Geniposide to evaluate the

potential of Geniposide and to consider it as a medication to lower blood pressure in the near future.

Materials and Methods

All the chemicals and reagents used were of analytical grade. Amphetamine and Hydralazine were purchased from Sigma-Aldrich, Mumbai, India. All drug solutions were freshly prepared in saline water before each experiment. Concentrated sulfuric acid, potassium bismuth iodide, Wagner reagent, Hagers reagents, sodium hydroxide, copper sulfate, hydrochloric acid, hydrogen peroxide, 4-aminoantipyrine, 4-chlorophenol, phosphotungstic acid, sodium dihydrogen phosphate, citric acid, potassium sulfate, and trichloracetic acid were purchased from Deshpande Laboratories Pvt Ltd, Bhopal, India.

Collection and authentication of plant material

The whole plant of *Paederia foetida* was collected and dried in 2022 from Bankura, West Bengal, India, with the GPS coordinates of 22°59′51.7"N 87°OO'39.4"E. The plant was authenticated by department of Botany, Bankura Christian College, West Bengal, India. The whole plant was shade-dried under room temperature and grinned to a fine powder.

Preparation of extraction

A whole plant powder (100 g) of *Paederia foetida* was extracted by maceration in 400 mL of ethanol for 14 days with frequent agitation. The mixture was filtered through clean muslin cloth followed by double filtration with Whatman No. 1 filter paper and the filtrate was concentrated by rotary evaporator with the vacuum at 50 °C, poured into glass Petri dishes, and brought to dryness at 60°C oven.

Phytochemical screening of different extract

Preliminary phytochemical screening was performed to identify the phytoconstituents present in the extracts. Phytochemical tests, such as Molish test, Dragendroff test, Mayers test, Wagner test, Hager test, Biuret test, Million test, ferric chloride test, lead acetate test, gelatin solution test, Shinoda test, alkaline reagent test, Legal test, Bajlets test, and Liebermann-Burchard test, were performed to determine the carbohydrates, alkaloids, proteins, tannins, flavonoids, glycosides, saponin, and steroids from ethanolic extract of *Paederia foetida.*

Table 1 Phytochemical screening of *Paederia foetida*

Serial no	Test	Result
1	Proteins and amino acids	+
2	Alkaloids	+
3	Phenolic compounds	+
4	Carbohydrates	+
5	Glycosides	+
6	Saponins	–
7	Flavonoids	+
8	Fixed oils and fats	–
9	Terpenoids	–
10	Sterols	–

The symbol (+) denotes presence and (–) denotes absence of phytoconstituents

Procurement of animals

The experiments were carried out according to the guidelines of the CPCSEA, New Delhi, India. The study protocol was approved by the Institutional Animal Ethical Committee of Aditya Bangalore Institute of Pharmacy Education and Research Bangalore, Karnataka, India (approval number: 64/1611/CPCSEA). Female Wistar rats (Wistar strain) weighing between 150 and 200 g were obtained from local vendor, Bangalore, Karnataka. Animals were housed into five groups under standard laboratory conditions, i.e., 25 °C ± 1 °C/45–55% RH and 12/12 h light and dark conditions in the animal house of Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, Karnataka, India. The rats are kept with free access to food (Hindustan Lever, India).

Antihypertensive activity

The rats were divided into five groups and each group contain six rats (n = 6). Group I was for positive control, received 0.9% saline p.o, group II marked for negative control, and received Amphetamine 5 mg/kg/day, (i.p.). Groups III and IV were allocated as test 1 and test 2 and received Amphetamine (5 mg/kg/day, i.p.) along with *Paederia foetida* (200 mg/kg/day, p.o.) and Amphetamine (5 mg/kg/day, i.p.) along with *Paederia foetida* (400 mg/kg/day, p.o.) for 4 weeks, respectively. Group V was marked as standard and received Amphetamine (5 mg/kg/day, i.p.) and Hydralazine (25 mg/kg., i.p.).

Blood pressure monitoring

Blood pressure was determined by placing an animal in the supplied restrainer. The tail-cuff warming chamber was attached and selected the number of test cycles and then pressed auto-calibrate to run. The tail-cuff method required the minimum amount of heat to measure blood pressure.

Noninvasive method of blood pressure determination

The cuff consists of latex balloon measuring 5 cm × 2 cm with 0.5 mm thickness. This balloon was placed in a circular plastic case having a diameter of 23 mm with a central hole of 12 mm diameter. The balloon was kept in

Table 2 SBP of Amphetamine-induced rat along with *Paederia foetida* and Hydralazine

Treatment groups (mg/kg)	Mean SBP (mm/Hg)				
	0 week	1st week	2nd week	3rd week	4th week
Control	108.32 ± 2.34*	109.43 ± 2.10*	114.12 ± 1.19*	112.23 ± 1.03*	110.15 ± 0.34*
Amphetamine (5)	108.21 ± 1.21*	116.14 ± 1.90*	131.01 ± 1.02*	139.57 ± 1.21*	148.07 ± 1.04*
Amphetamine (5) + *Paederia foetida* whole plant extract (200)	109.10 ± 0.19*	114.27 ± 1.54	125.32 ± 1.23*	122.27 ± 0.91*	119.37 ± 0.56*
Amphetamine (5) + *Paederia foetida*(P.F.) whole plant extract (400)	108.45 ± 1.11*	112.65 ± 0.65*	120.54 ± 0.45*	119.68 ± 1.02*	116.67 ± 0.34*
Amphetamine (5) + Hydralazine (25)	109.27 ± 0.21*	111.91 ± 2.34*	118.06 ± 1.23*	115.34 ± 0.22*	112.38 ± 0.12*

Values are expressed as mean ± std, n = 6. Differences were considered significant at *P value of < 0.05. For numerical results, analysis of variance (ANOVA) was performed using GraphPad InStat version 3

* indicates level of significance

Table 3 Effect of *Paederia foetida* whole plant extract (200, 400 mg/kg/day, p.o., for 4 weeks) on serum sodium value, in Amphetamine-induced hypertensive rats and taking Hydralazine as a standard

Treatment groups(mg/kg)	Serum Na + (mMol/ml)			
	1st week	2nd week	3rd week	4th week
Control	152.23 ± 0.18*	152.47 ± 0.19*	153 ± 0.27*	153.42 ± 0.44*
Amphetamine (5)	151.40 ± 0.48*	150.85 ± 0.44*	149.85 ± 0.97*	148.83 ± 0.46*
Amphetamine (5) + *Paederia foetida* (P.F.) (200)	151.31 ± 0.30*	151.55 ± 0.48*	151.81 ± 0.22*	151.29 ± 0.37*
Amphetamine (5) + *Paederia foetida* (P.F.) (400)	151.39 ± 0.89*	151.66 ± 0.55*	151.96 ± 0.87*	152.09 ± 0.17*
Amphetamine (5) + Hydralazine (25)	152 ± 0.02*	152.11 ± 0.69*	152.11 ± 0.88*	154.84 ± 0.54*

Values are expressed as mean ± std, n = 6. Differences were considered significant at *P value of < 0.05. For numerical results, analysis of variance (ANOVA) was performed using GraphPad InStat version 3

* indicates level of significance

Table 4 Effect of *Paederia foetida* whole plant extract (200, 400 mg/kg/day, p.o., for 4 weeks) on serum potassium value, in Amphetamine-induced hypertensive rats and taking Hydralazine as a standard

Serum K^+(mMol/ml)				
Treatment groups (mg/kg)	**1st week**	**2nd week**	**3rd week**	**4th week**
Normal control	5.15 ± 0.17**	5.29 ± 0.09**	5.31 ± 0.09**	5.26 ± 0.13**
Amphetamine (5)	5.29 ± 0.16**	5.38 ± 0.07**	5.49 ± 0.10**	5.36 ± 0.10**
Amphetamine (5) + *Paederia foetida*(P.F.)(200)	5.26 ± 0.11**	5.33 ± 0.13**	5.39 ± 0.03**	5.36 ± 0.10**
Amphetamine (5) + *Paederia foetida*(P.F.)(400)	5.23 ± 0.17**	5.42 ± 0.14**	5.36 ± 0.15**	5.32 ± 0.09**
Amphetamine (5) + Hydralazine(25)	5.20 ± 0.03**	5.27 ± 0.04**	5.23 ± 0.06**	5.24 ± 0.00**

Values are expressed as mean ± std, n = 6. Differences were considered significant at *P value of < 0.05. For numerical results, analysis of variance (ANOVA) was performed using GraphPad InStat version 3

** indicates level of significance of ANOVA result

such a manner that it remains in contact with the inner surface of the plastic case around the central hole so that this balloon encircles the tail. One end of the triway was connected with the balloon (tail-cuff) and the other two ends were connected to an inflating–deflating pump and sphygmomanometer. The system measured the systolic BP by determining the cuff pressure (reflected on the sphygmomanometer) and the blood flow (pulse) to the tail was eliminated. This elimination of blood flow (pulse) was recorded by a pulse transducer connected to a single-channel physiograph through a suitable coupler. The animals were acclimatized and kept in restrainers. The tail was passed through the hole of the newly designed cuff and the pulse transducer was tied around the tail distal to the cuff. As the system was switched on and the pulse was recorded on the physiographic paper.

Table 5 Binding energy of Geniposide and Estradiol with estrogen receptor

Compounds	PDB ID	S(Kcalmol^{-1})	Amino acids	Interaction	Length	Figure No
Geniposide	3OLL	-7.67	ARG346	(Lig.) O…..H (ARG346)	2.95	1(a)
			LEU298	(Lig.) O…..H (LEU298)	2.66	
			GLU305	(Lig.) O…..H (GLU305)	2.52	
			HIS475	(Lig.) O…..H (HIS475)	3.18	
Estradiol		-9.93	HIS475	(Lig.) O…..H (HIS 475)	3.03	1(b)
			ARG346	(Lig.) O…..H (ARG 346)	3.09	
Geniposide	3OLS	-8.91	HIS475	(Lig.) O…..H (HIS 475)	3.10	1(c)
			LEU339	(Lig.) H…..O (LEU 339)	3.33	
			GLU305	(Lig.) H…..O (GLU 305)	2.76	
			ARG346	(Lig.) H…..O (GLU 305)	2.34	
			LEU298	(Lig.) O…..H (ARG 346)	3.07	
				(Lig.) O…..H (LEU 298)	2.46	
Estradiol		-10.91	HIS475	(Lig.) O…..H (HIS 475)	3.10	1(d)
			ARG346	(Lig.) O…..H (ARG 346)	3.17	
Geniposide	5DX3	-7.33	HIS524	(Lig.) O…..H (HIS 524)	3.13	2(a)
			LEU346	(Lig.) O…..H (LEU 346)	2.37	
			ALA350	(Lig.) O…..H (ALA 350)	3.07	
			GLU353	(Lig.) O…..H (GLU 353)	3.17	
			LEU387	(Lig.) O…..H (GLU 353)	2.17	
			ARG394	(Lig.) O…..H (LEU 387)	3.14	
				(Lig.) O…..H (ARG 394)	2.93	
Estradiol		-10.61	HIS524	(Lig.) O…..H (HIS 524)	3.11	2(b)
			ARG394	(Lig.) O…..H (ARG 394)	2.98	
Geniposide	5DXE	-8.39	HIS524	(Lig.) O…..H (HIS 524)	3.06	2(c)
			LEU346	(Lig.) O…..H (LEU 346)	2.38	
			GLU353	(Lig.) O…..H (GLU 353)	2.39	
			LEU387	(Lig.) O…..H (LEU 387)	2.90	
			ARG394	(Lig.) H…..O (ARG 394)	2.94	
Estradiol		-10.88	HIS524	(Lig.) O…..H (HIS 524)	3.07	2(d)
			ARG394	(Lig.) O…..H (ARG 394)	2.98	
Geniposide	6PIT	-8.21	HIS524	(Lig.) O…..H (HIS 524)	3.08	2(e)
			LEU346	(Lig.) O…..H (LEU 346)	2.41	
			GLU353	(Lig.) O…..H (GLU 353)	2.29	
			ARG394	(Lig.) O…..H (GLU 353)	3.31	
			LEU387	(Lig.) O…..H (ARG 394)	2.92	
				(Lig.) O…..H (LEU 387)	2.95	
Estradiol		-10.53	ARG394	(Lig.) O…..H (ARG 394)	3.09	2(f)

The cuff was inflated by the pump and the pressure in the cuff was raised until the pulse was eliminated. The pressure at which the pulse was eliminated, and noted as the systolic BP of the animal. The systolic BP and pulse were also recorded by the NIBP system of ADI for comparison.

Estimation of electrolytes

Method for assessment of serum electrolyte

Wistar rats were used for the biochemical analysis of serum. The rats were provided with the usual diet and the blood samples were collected for the determination of the amount of electrolytes present in blood (Na^+, K^+).

Sodium and potassium ion estimation

Sodium and potassium determination was performed in an automated Roche 9180 electrolyte analyzer (Basel, Switzerland). The 2 ml of blood samples was collected from rats through retro-orbital plexus after anesthetizing through a diethyl ether inhaler. The blood samples were kept for 30 min then centrifuged in a cooling centrifuge at 5000 rpm for 10 min to obtain the serum. The calibration of the instrument was performed, then run a control sample as mentioned. Finally, the serum sample was run to obtain the results.

In silico analysis

Ligand and protein preparations

The selected compound Geniposide and Estradiol (Internal ligand) were utilized for the molecular docking analysis for estrogen receptors. The three-dimensional structures of Geniposide and Estradiol were acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) (accessed on 20 April 2023) in SDF MOL format. The SDF MOL format was converted into PDB format by using OpenBableGUI 3.1.1 software. Three-dimensional structures (mainly crystal) of different PDB ID like 3OLL

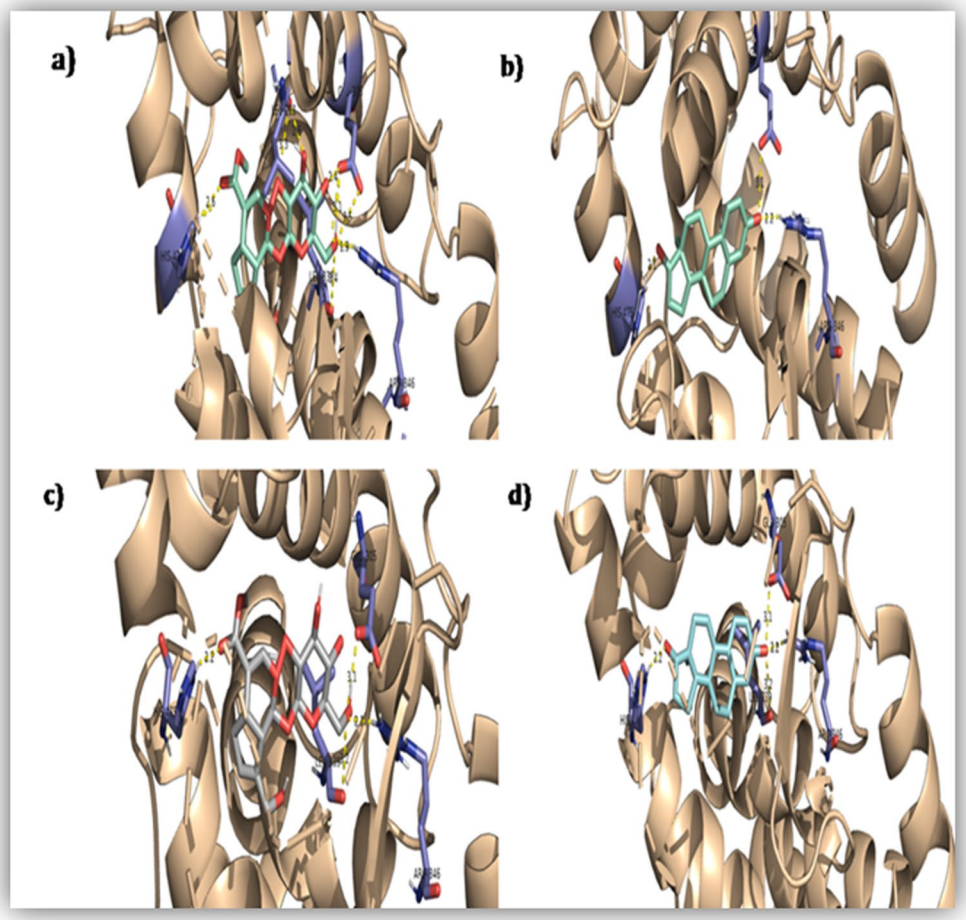

Fig. 1 3D Molecular interaction of: **a** Geniposide and **b** Estradiol with 3OLL and **c** Geniposide and **d** Estradiol with 3OLS

[40], 3OLS [40], 5DX3 [41], 5DXE [41], and 6PIT [42] were chosen for the investigation acquired from RCSB Protein Data Bank (PDB). 3OLL-Crystal structure of phosphorylated estrogen receptor beta ligand binding domain, 3OLS-Crystal structure of phosphorylated estrogen receptor beta ligand binding domain, 5DXE-Estrogen Receptor Alpha Ligand Binding Domain Y537S Mutant in Complex with Stapled Peptide SRC2-P4 and Estradiol, 5DX3-Estrogen Receptor Alpha Ligand Binding Domain Y537S Mutant in Complex with Stapled Peptide SRC2-P3 and Estradiol & 6PIT-Estrogen Receptor Alpha Ligand Binding Domain Y537S Mutant in Complex with SRC2 Stapled Peptide 41A and Estradiol. The process of getting ready the proteins involved accessing the PDB ID to retrieve the protein from the server, uploading the molecule, adding hydrogens, applying specific turning to residues, examining interactions and geometry for all atoms, and acquiring the finished protein file. Estradiol was isolated from the protein's binding pocket as an internal ligand to reveal the grid coordinates throughout the active region. The stabilized structure was stored after being downloaded from the server in PDBQT format for the purpose of protein–ligand docking.

Molecular docking of protein and ligand

The Autodock-4.2.6 program (ADP) was used to carry out molecular docking of Geniposide & Estradiol with estrogen receptors. ADP tools were used to prepare the protein and ligands. The coordinate values used in grid settings were acquired from re-docking studies, and the dimensions of the grid box were 60×60x60 in the x, y, and z directions. In each case, the spacing of the grid point was 0.375". Auto grid-4.2 was utilized to create the map files. For search criteria, a genetic algorithm (GA) was employed. Autogrid and Autodock operation were the last step of docking analysis and were performed in Autodock-4.2.6. The molecular docking of individual ligands on the appropriate protein was carried out using Autodock-4.2 and Autogrid-4.2, respectively. Interactions

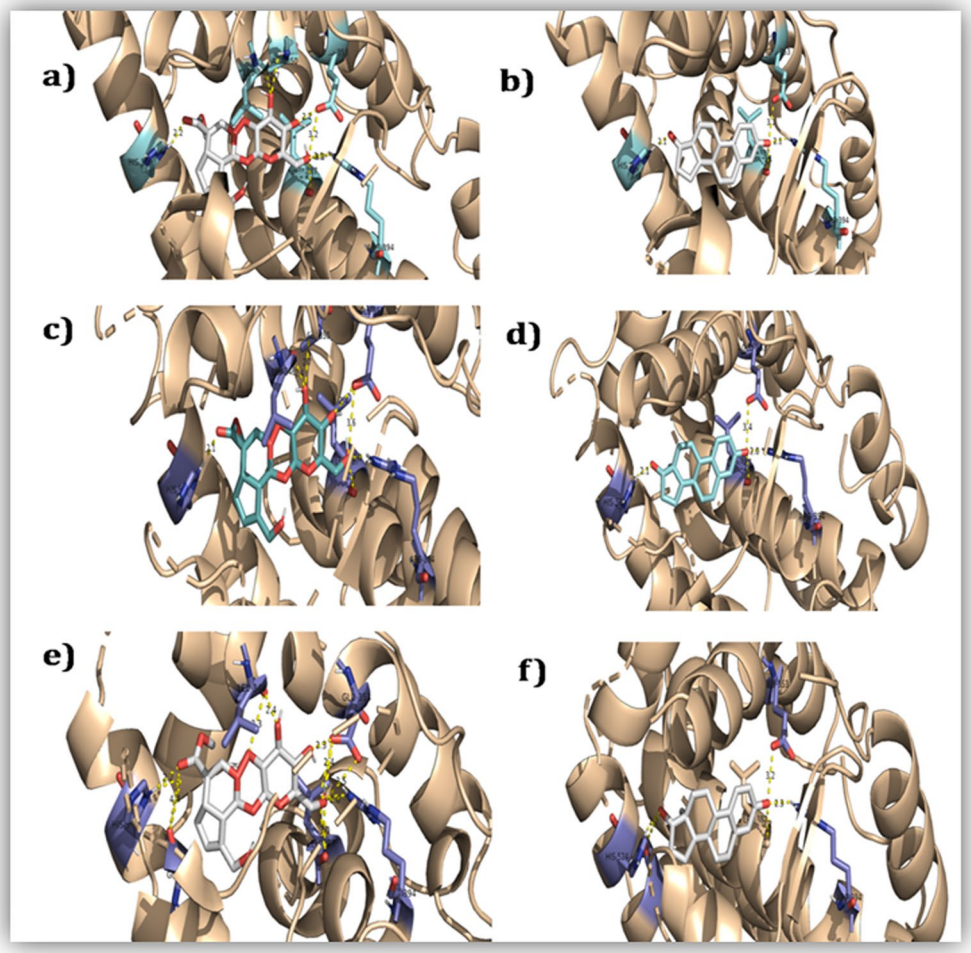

Fig. 2 3D Molecular interaction of: **a** Geniposide and **b** Estradiol with 5DX3, **c** Geniposide and **d** Estradiol with 5DXE and **e** Geniposide and **f** Estradiol with 6PIT

between molecules and binding energy (kcal/mol) were measured and analyzed.

ADME profile

The Swiss ADME online server (http://www.swissadme.ch/index.php) was used to assess the drug-likeness patterns and pharmacokinetic characteristics of the investigated compounds, where SMILES format of the compounds was uploaded to the webpage and the assessment procedure was completed.

Toxicity profile

The ProTox-II online server (https://tox-new.charite.de/protox_II/index.php) was used to predict the toxicities of the investigated compounds, where SMILES format of the compounds was uploaded to the webpage and the assessment procedure was completed.

Molecular dynamic simulation

Molecular dynamics (MD) simulation was carried out using GROMACS 2022.2. The following steps were utilized.

(a) Preparation of enzyme

The 3-dimensional (3D) models of the Estrogen receptor in complex with Geniposide were exported to PDB format using Pymol. The dynamic behavior of the complex was evaluated using molecular dynamic (MD) simulation in the GROMACS package program (version 2022.2) [43–45]. Protein topology was constructed

Table 6 ADME properties of the selected compounds predicted using SwissADME web server

Property	Geniposide	Estradiol
MW	388.37	272.38
Consensus LogPo/w	-1.25	3.40
Log *S* (ESOL)	Very soluble	Moderately soluble
#Rotatable bonds	6	0
#H-bond acceptors	10	2
#H-bond donors	5	2
MR	86.89	81.03
TPSA	155.14	40.46
Lipinski violations	0	0
Ghose violations	1	0
Veber violations	1	0
Egan violations	1	0
Muegge violations	2	0
Bioavailability score	0.11	0.55
PAINS alerts	0	0
Brenk alerts	1	0
Leadlikeness violations	1	1
GI absorption	Low	High
BBB permeant	No	Yes
P-gp substrate	No	Yes
CYP1A2 inhibitor	No	No
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	Yes
CYP3A4 inhibitor	No	No

by pdb2gmx with the CHARMM27 force field [46], and ligand topology was generated using the SwissParam server [47].

(b) Setting up a system for simulation

After applying the force field, the complex was inserted into the system. It was solvated with the TIP3P water model [48] in a cubic box greater than 1 nm from the edge of the protein with periodic boundary conditions. The system was neutralized by adding Na+ions, and energy minimization was done for 50,000 steps using the steepest descent algorithm. It was then followed by 100 ps of NVT simulation at 300 K and 100 ps of NPT simulations to equilibrate the whole system. The Leap-frog algorithm was employed in the constant-temperature, constant-pressure (NPT) ensemble to separately couple each component like protein, ligand, water molecules, and ions [49]. The Berendsen temperature and pressure coupling constants were set to 1 and 2, respectively, to keep the system in a stable environment (300 K temperature and 1 bar pressure) [50]. Finally, MD simulation for 100 nswas performed in isothermal and isobaric condition ensemble at 300 K. The pressure coupling with time-constant was set at 1 ps to maintain pressure constant at 1 bar, and the LINCS algorithm [51] was used to constrain the bond lengths. The Van der Waals and Coulomb interactions were truncated at 1.2 nm, and the PME algorithm [52] inbuilt into GROMACS was used to minimize the error from truncation.

(c) Visualization and analysis of simulation

The trajectory file was visualized through VMD (Visual Molecular Dynamics) 1.9.2 [53] and analyzed by the tool Hero MD Analysis [54, 55] and Xmgrace 5.1.25 [56, 57].

Results

Preparation of extract and phytochemical screening

The crude ethanolic extract was prepared by maceration process for a period of 14 days and finally the yield was calculated. Nearly 100 gm of the whole plant was extracted and the yield was found to be 12.16 g. An appropriate concentration of the extracts was made in distilled water for further studies. Preliminary phytochemical screening of ethanolic extract of *Paederia foetida* was performed and revealed the presence of different phytoconstituents, proteins, amino acids, alkaloids, phenolic compounds, carbohydrates, alkaloids, tannins, flavonoids, and glycosides. The results of all these tests are summarized in Table 1.

Measurement of blood pressure by noninvasive (indirect) method after administration of Paederia foetida whole plant extract

The drug-induced hypertension in rats was acquired by administration of Amphetamine for 4 weeks (negative control) in rats, and produced a significant elevation in the systolic blood pressure (SBP) as measured by tail-cuff method on 1st, 2nd, 3rd, and 4th week, respectively, when compared to the control rats. Rats that received *Paederia foetida* whole plant extract (200 and 400 mg/kg/day, p.o., test 1 & 2) for 4th weeks along with Amphetamine was found to reduce the SBP significantly in 3rd and 4th week when compared to Amphetamine-induced hypertensive rats, thus implying an antihypertensive activity. The detailed antihypertensive study is summarized in Table 2 and Additional file 1: Figure S1.

Estimation of sodium and potassium ion in serum of rats

The blood sample from different groups was collected and serum was separated to carry out the estimation of sodium and potassium levels in the serum of rats. The normal control (Group I) test 1, (Group III), test 2 (Group IV) & standard (Group V) exhibited normal values of sodium, whereas the negative control group (administered with Amphetamine) showed a relative decrease in Na^+ level in blood serum for 4 weeks in rats.

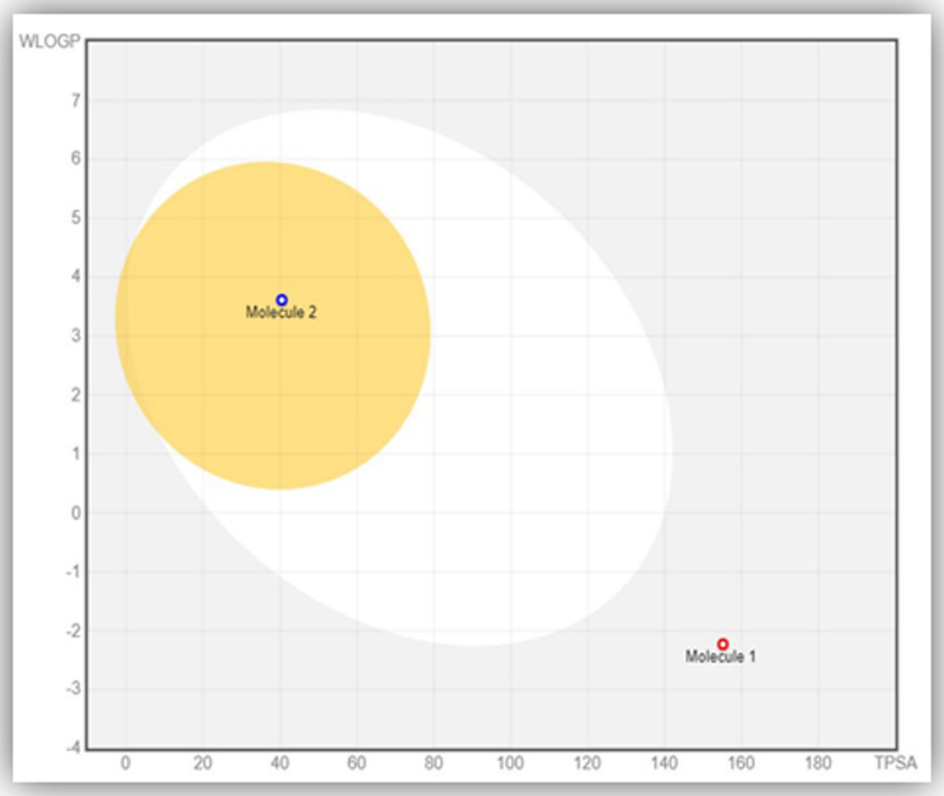

Fig. 3 The boiled egg chart of the compounds: Geniposide (Molecule 1) and Estradiol (Molecule 2)

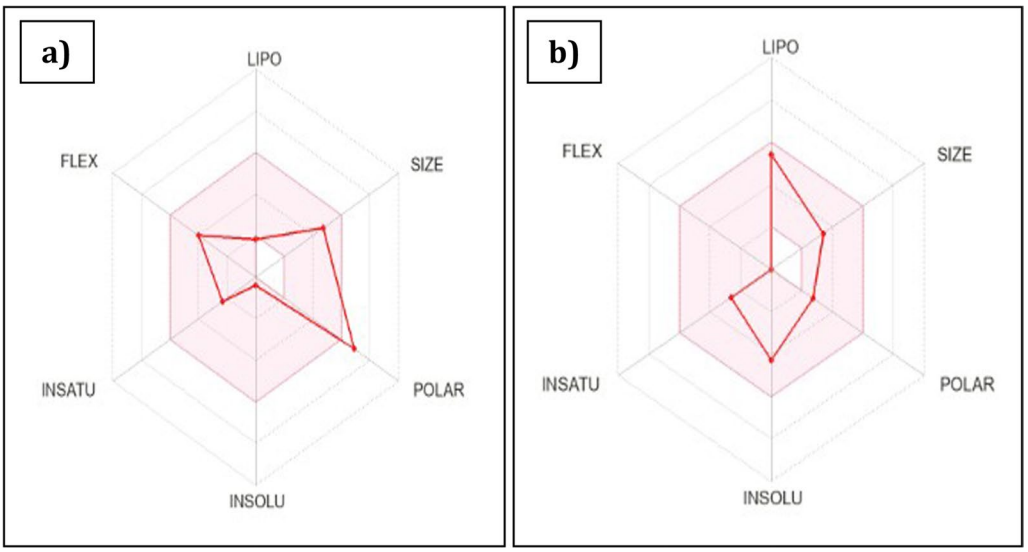

Fig. 4 Bioavailability radar chart of the compounds; **a** Geniposide and **b** Estradiol. The pink area demonstrates the range of the optimal property values for oral bioavailability and the red line is the compounds' predicted properties. Saturation (INSATU), size (SIZE), polarity (POLAR), solubility (INSOLU), lipophilicity (LIPO), and flexibility (FLEX)

Table 7 Toxicity model report of Geniposide

Classification	Target	Prediction	Probability
Organ toxicity	Hepatotoxicity	Inactive	0.84
Toxicity end points	Carcinogenicity	Inactive	0.85
Toxicity end points	Immunotoxicity	Active	0.52
Toxicity end points	Mutagenicity	Inactive	0.63
Toxicity end points	Cytotoxicity	Inactive	0.66
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon receptor (AhR)	Inactive	0.96
Tox21-Nuclear receptor signaling pathways	Androgen receptor (AR)	Inactive	0.94
Tox21-Nuclear receptor signaling pathways	Androgen receptor ligand binding domain (AR-LBD)	Inactive	0.9
Tox21-Nuclear receptor signaling pathways	Aromatase	Inactive	0.9
Tox21-Nuclear receptor signaling pathways	Estrogen receptor alpha (ER)	Inactive	0.68
Tox21-Nuclear receptor signaling pathways	Estrogen receptor ligand binding domain (ER-LBD)	Inactive	0.97
Tox21-Nuclear receptor signaling pathways	Peroxisome proliferator activated receptor gamma (PPAR-Gamma)	Inactive	0.94
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	0.96
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive	0.96
Tox21-Stress response pathways	Mitochondrial membrane potential (MMP)	Inactive	0.79
Tox21-Stress response pathways	Phosphoprotein (tumor suppressor) p53	Inactive	0.9
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	Inactive	0.96

Table 8 Toxicity model report of Estradiol

Classification	Target	Prediction	Probability
Organ toxicity	Hepatotoxicity	Inactive	0.72
Toxicity end points	Carcinogenicity	Inactive	0.84
Toxicity end points	Immunotoxicity	Active	0.99
Toxicity end points	Mutagenicity	Inactive	0.98
Toxicity end points	Cytotoxicity	Inactive	0.87
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon receptor (AhR)	Inactive	1
Tox21-Nuclear receptor signaling pathways	Androgen receptor (AR)	Active	1
Tox21-Nuclear receptor signaling pathways	Androgen receptor ligand binding domain (AR-LBD)	Active	1
Tox21-Nuclear receptor signaling pathways	Aromatase	Inactive	0.98
Tox21-Nuclear receptor signaling pathways	Estrogen receptor alpha (ER)	Active	1
Tox21-Nuclear receptor signaling pathways	Estrogen receptor ligand binding domain (ER-LBD)	Active	1
Tox21-Nuclear receptor signaling pathways	Peroxisome proliferator activated receptor gamma (PPAR-Gamma)	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	0.98
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive	0.98
Tox21-Stress response pathways	Mitochondrial membrane potential (MMP)	Active	1
Tox21-Stress response pathways	Phosphoprotein (tumor suppressor) p53	Active	1
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	Inactive	1

Our findings also satisfy the literature survey as it was mentioned by Suzanne et al. 2002 [39] that Amphetamine toxicity can relatively decrease the Na^+ level than the normal value in blood serum. The result of the study is represented in Table 3 and Additional file 1: Figure S2. In the case of the serum potassium level in rats, no significant changes were noticed in all the four groups (Table 4 and Additional file 1: Figure S3).

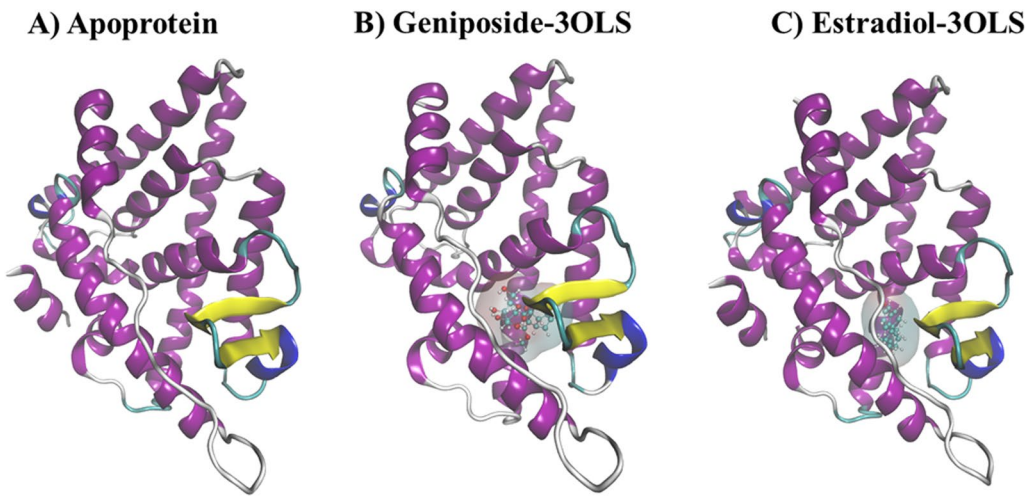

Fig. 5 Graphical representation of **A** Apoprotein form, **B** Geniposide and **C** Estradiol bound inside the active site of Estrogen receptor, where protein is shown in cartoon representation and the ligand is shown in CPK representation

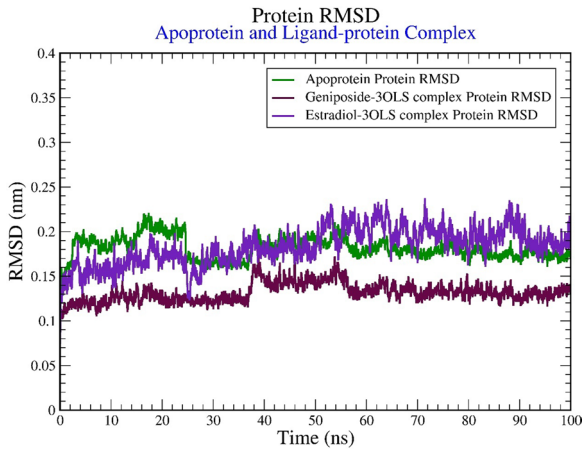

Fig. 6 Graphical representation of the plots showing protein Cα RMSD (nm) versus time (100 ns) for different models

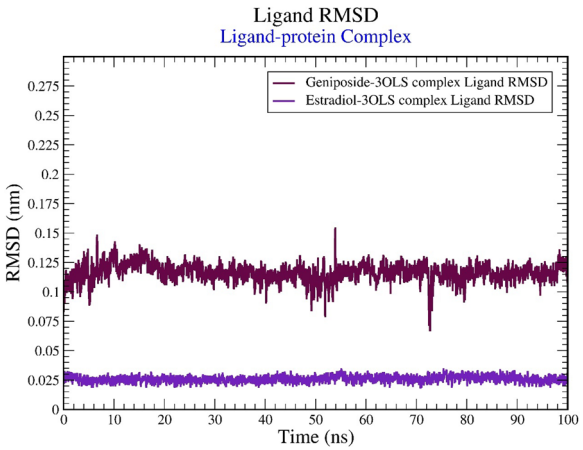

Fig. 7 Graphical representation of the plots showing ligand RMSD (nm) versus time (100 ns) for Geniposide-3OLS and Estradiol-3OLS complex

In silico activity of Geniposide and Estradiol against estrogen receptor

Molecular docking of Geniposide and Estradiol

The literature survey reveals that Geniposide is an active ingredient which can effectively reduce the blood pressure by inhibiting the activation of WNK signaling pathway mediated by the estrogen receptor [14]. The goal of the molecular docking study was to examine at the binding energy and molecular interactions of Geniposide and Estradiol in the active site of the estrogen receptor, to achieve a strong rational correlation through computational studies. In addition, each compound's binding mechanism was examined for molecular interactions. The results showed that Geniposide had very close significant binding energy compared to the internal ligand Estradiol (Table 5). Among all the interactions (3OLS, 3OLL, 5DX3, 5DXE, 6PIT), PDB ID: 3OLS, Geniposide was found to have a very good docking score. The result of the binding and molecular interaction between PDB ID: 3OLS & Geniposide (Fig. 1c) revealed that Geniposide formed hydrogen bonds with HIS475, GLU3O5, LEU298, LEU339, and ARG346 through oxygen groups having bond lengths of 3.10, 2.76, 2.46, 3.33 & 3.07 Å, respectively. In the case of internal ligand Estradiol, it showed hydrogen bond interaction between HIS475 & ARG346 through oxygen atoms having bond lengths of 3.10 & 3.17 Å, respectively (Fig. 1d). All other interactions are shown

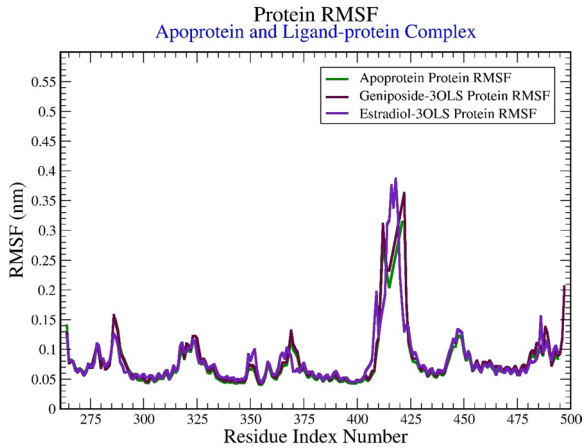

Fig. 8 Graphical representation of the plots showing protein RMSF (nm) versus residue index number of protein for different models

in Fig. 2. 2D molecular interactions of Geniposide and Estradiol with respect to all PDB ID are given in Additional file 1 (Figure S4- Figure S8).

ADME profile

The pharmacokinetics, physicochemical, and drug-likeness parameters of the compounds Geniposide (Plant derived) & Estradiol (Internal Ligand) were evaluated using the freely available online server Swiss ADME (http://www.swissadme.ch/index.php). The overall results of Swiss ADME prediction (Table 6) elucidated that the examined compounds showed a predicted log Po/w value in the range of -1.25 to 3.40 and disclosed no alerts for Pan Assay Interfering Substances (PAINS). Geniposide has poor solubility in water, but Estradiol was practically insoluble in water. Geniposide does not show BBB permeability, but Estradiol shows BBB permeability. This parameter was well understood from the boiled egg chart (Fig. 3). Both of these compounds follow Lipinski rule of five. Estradiol was found to be a CYP2D6 inhibitor, whereas Geniposide does not inhibit the enzyme CYP2D6 that suggests that Geniposide does not interfere with the metabolism of other drugs associated with CYP2D6. Lastly, the oral bioavailability radar charts (Fig. 4) showed good oral bioavailability as it is polar in nature, whereas Estradiol is nonpolar in nature. This result reflects Geniposide a favorable compound for good pharmacokinetic features. Along with this features, Geniposides also possess greater flexibility, lesser lipophilicity, and higher solubility when compared to Estradiol. This proves that Geniposide is having better ADME profile than Estradiol.

Toxicity profile

The toxicity parameters of Geniposide (Plant derived) & Estradiol (Internal Ligand) were evaluated using the online server ProTox-II (https://tox.new.charite.de/protox_II/index.php) and the result is depicted in Tables 7 and 8. It was observed that Geniposide is safer in comparison with Estradiol and it is clearly understood from the toxicity radar chart and toxicity table that all the toxicity parameters were inactive except immunotoxicity in

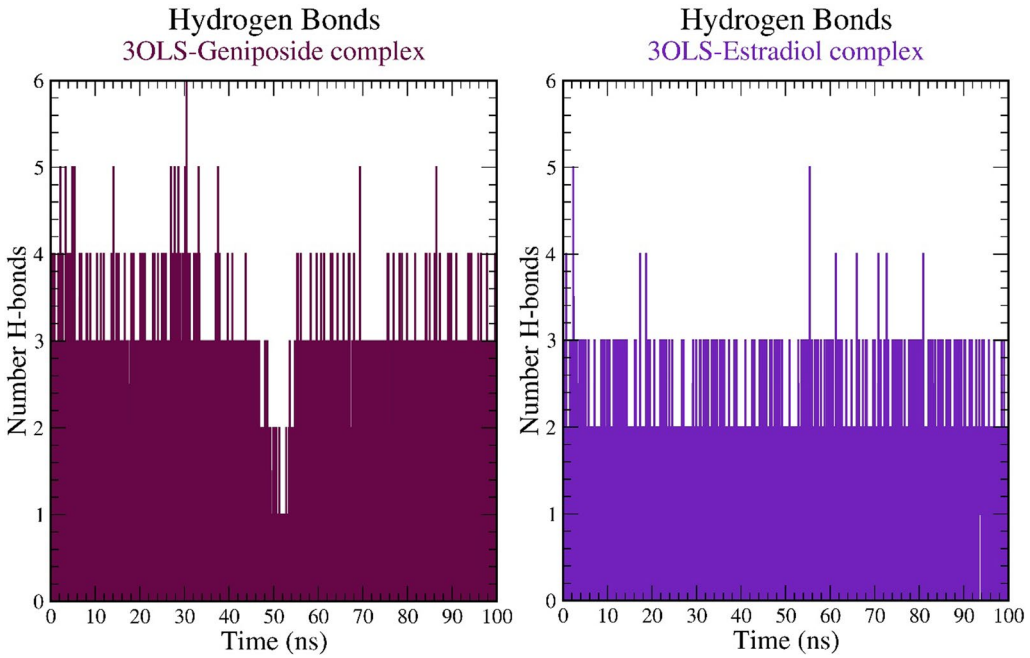

Fig. 9 Pictorial representation of the number of h-bond contacts formed by Geniposide and Estradiol in complex with Estrogen receptor (3OLS)

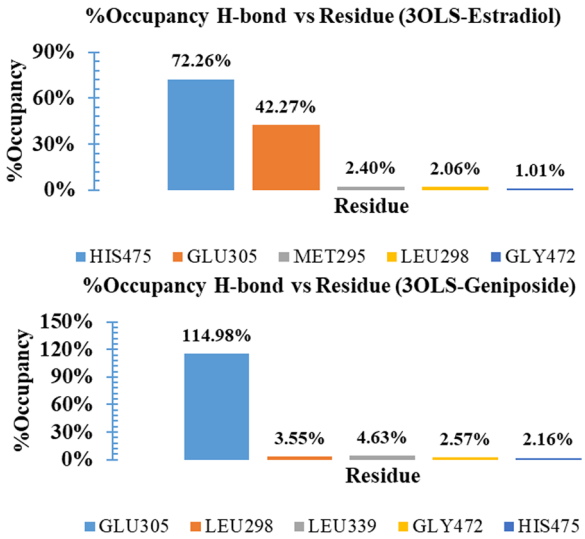

Fig. 10 Histogram representation of %occupancies of the h-bond protein ligand contacts of Estradiol and Geniposide in complex with Estrogen receptor (3OLS)

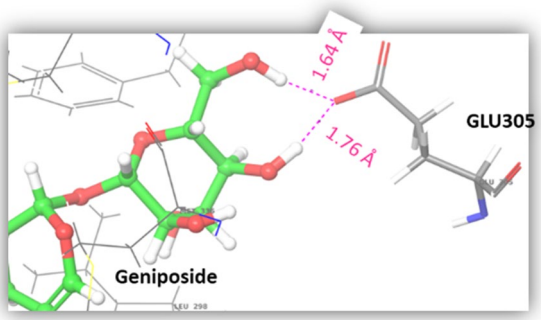

Fig. 11 Representation of multiple h-bond contact by Geniposide with residue GLU305 of Estrogen receptor

case of Geniposide, whereas several toxicity parameters were found active in Estradiol. The immunotoxicity of Geniposide was found to be 0.52, whereas that of Estradiol was 0.99. This refers that Geniposide is safer option than that of Estradiol. Geniposide is much safer compare to Estradiol for the target androgen receptor, from the toxicity profile study of both these drugs depleted in Additional file 1: Figure S9.

Molecular dynamic simulations of estrogen receptorin Apoprotein form and in complex with molecules, Geniposide and Estradiol

In order to evaluate the binding of molecule, Geniposide and Estradiol inside the β-ligand binding domain of Estrogen receptor, we have carried out MD simulations for a period of 100 ns for the three models, A) Apoprotein, B) Geniposide-3OLS, and C) Estradiol-3OLS complex (Fig. 5). Their simulations were evaluated using various statistical parameters including: root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), h-bond interactions, and its %occupancies over the time.

RMSD analysis

Analyzing the protein-RMSD can give insights into any structural conformation that protein undergoes during the simulation. The multiplot for protein Cα and ligand RMSD versus time is shown in Figs. 6 and 7, respectively. The protein CαRMSD plot indicates that the Apoprotein and the complexes (protein and ligand) have thoroughly followed the equilibrium RMSD value of around 0.17 nm.

The ligand RMSD plot revealed the stability of both Geniposide and Estradiol in their binding to the estrogen receptor during the entire simulation. Geniposide and Estradiol achieved RMSD values of approximately 0.1 nm and 0.025 nm, respectively.

RMSF analysis

Protein RMSF proves valuable for assessing local changes in the protein chain. The multiplot for protein RMSF (nm) versus residue number index is depicted in Fig. 8. Encouragingly, the plot indicates fluctuations of less than 0.15 nm for most of the protein sequence, with the exception of the loop region between ASN407-SER422 in all three cases—Apoprotein, Geniposide-3OLS, and Estradiol-3OLS. Although this loop region exhibits fluctuations of around 0.35 nm, it does not impact ligand binding, as no residue in this region contributes to the ligand's binding cavity. The residues within the binding cavity remain stable with fluctuations of significantly lower magnitude.

H-bond interaction

Molecular interactions, especially h-bond interactions, are sensitive to distance and angle variations, susceptible to disruption under dynamic conditions. In this study, we scrutinized the ligand–protein interactions in both complexes. Figure 9 presents the plot of the number of hydrogen bonds versus time. Notably, Geniposide exhibited up to three stable h-bond contacts during the simulation, while Estradiol displayed up to two stable contacts.

Figure 10 illustrates the %occupancies histogram of h-bond contacts formed by the ligand Geniposide in complex with the Estrogen receptor. The most stable interaction was observed with residue GLU305, exhibiting an occupancy of 114.98%. This indicates that the

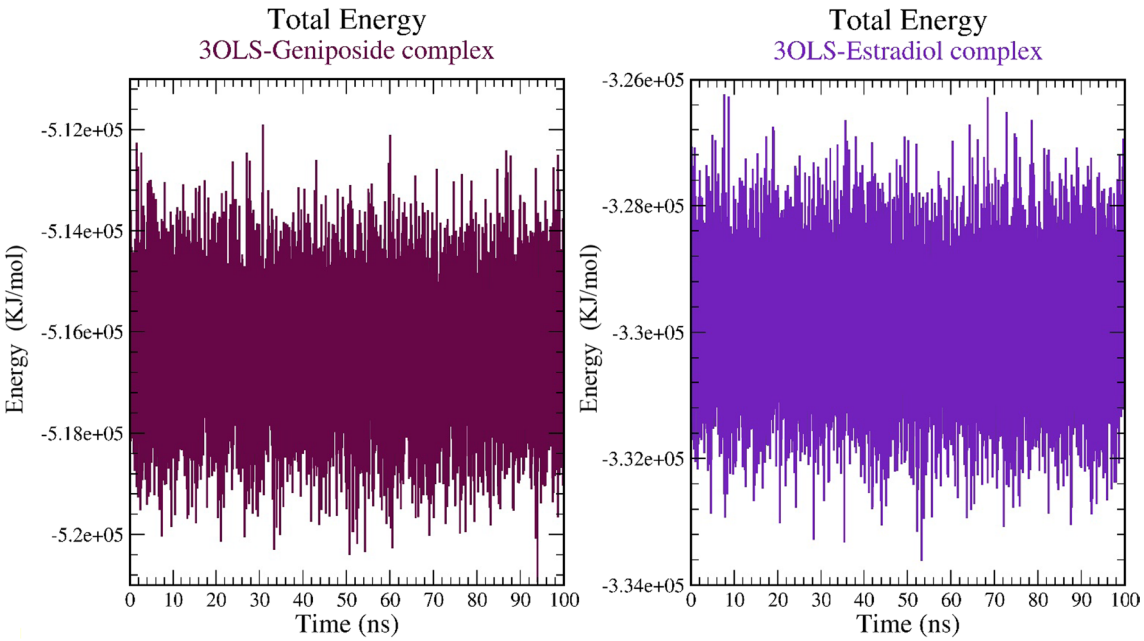

Fig. 12 Graphical representation of the total energy (KJ/mol) versus time (100 ns) for 3OLS-Geniposide and 3OLS-Estradiol complex

ligand established multiple stable h-bond contacts with this residue (see Fig. 11), while Geniposide weakly interacted with other residues, including LEU298, LEU339, GLY472, and HIS475, with %occupancies of 3.55, 4.63, 2.57, and 2.16%, respectively. In comparison, the ligand Estradiol interacted with residues HIS475 and GLU305, with occupancies of 72.26% and 42.27%, respectively. Other residues, such as MET295, LEU298, and GLY472, exhibited interactions with occupancies of 2.4%, 2.06%, and 1.01%, respectively. In summary, similar to Estradiol, Geniposide efficiently activates the Estrogen receptor, demonstrating effective binding within the β-ligand domain. Estrogen receptor activation is known to promote vasodilation and exert antihypertensive effects by modulating the renin–angiotensin–aldosterone system [58].

Total energy

The total energy profiles obtained from the MD simulations of the Geniposide-3OLS and Estradiol-3OLS complexes offer valuable insights into their stability and energetic characteristics. Across the 100 ns simulation period, both complexes displayed minor fluctuations in total energy, indicating the attainment of a relatively stable equilibrium state (Fig. 12). These fluctuations remained within acceptable ranges, indicative of a well-equilibrated simulation that faithfully captured the dynamic behavior of the complexes.

Discussion

Hypertension is also regarded as the main risk factor for disability-adjusted life years globally, according to the Global Burden of Disease study [2]. Numerous research are focused on development of novel antihypertensive medicines and new therapy options because of the massive prevalence of hypertension, worldwide [4, 5]. Recently, it has been revealed that medicinal herbs can reduce hypertension and can be used empirically as antihypertensive medicines [8, 9]. Diuretics have a significant role in the clinical management of hypertension. These medications influence the renal tubular segments' salt re-absorption to boost kidney urine production [12, 13]. NCC (Sodium chloride co-transporters) and NKCC2 (Na–K-2Cl co-transporter) are the main targets of diuretics. Recent research has demonstrated that the NKCC2-oxidative stress-responsive kinase 1 (OSR1)-with-no-lysine kinase (WNK) signaling pathway is crucial for controlling blood pressure [14]. A Rubiaceae family member *Paederia foetida*, also known as Prasariniin in Sanskrit, is a climbing plant with a strong foetid odor. Its Hindi name is Gandhaprasarini, and its English name is Chinese Flower [17]. Various types of phytochemicals are present in the *Paederia foetida*, among these one Iridoid glycosides known as Geniposide present in the root of this plant [33]. Yang Fu et al. [14] demonstrated that the ability of Geniposide to increase urine production and the excretion of salt and chloride ions. They further revealed that

the antihypertensive effects of Geniposide was considered due to their inhibition of the activation of the WNK signaling pathway, which is mediated by the estrogen receptor. So, considering this key finding we performed molecular docking and molecular dynamics (MD) simulations studies of Geniposide and various PDB IDs based on the structure of estrogen receptor. *Paederia foetida* whole plant extract has an antihypertensive action in Amphetamine-induced hypertensive rats which is evident by a considerable decrease in blood pressure. Amphetamine induces decreasing sodium level that can be countered by *Paederia foetida* whole plant extract, whereas sodium & potassium level remains to be same in all the four groups of rats. The goal of the molecular docking study was to examine the binding energy and molecular interactions of Geniposide and Estradiol in the active site of the estrogen receptor, to achieve a strong rational correlation through computational studies. In addition, each compound's binding mechanism was examined for molecular interactions. The results showed that Geniposide had very close significant binding energy compared to the internal ligand Estradiol. The result of ADME study reflects that Geniposide is a favorable compound for good pharmacokinetic features. Along with these features, Geniposides also possess greater flexibility, lesser lipophilicity, and higher solubility when compared to Estradiol. This proved that Geniposide is having better ADME profile than Estradiol. Geniposide is safer in comparison with Estradiol and it is clearly understood from the toxicity radar chart and toxicity table that all the toxicity parameters were inactive except immunotoxicity in case of Geniposide, whereas several toxicity parameters were found active in Estradiol. The molecular dynamics revealed that Geniposide forms stable interactions with key amino acid, GLU305 of β-ligand binding domain of estrogen receptor, suggesting its potential as an activator of estrogen receptor. After overall evaluation of all the data, we can say that Geniposide is a potential drug candidate for the treatment hypertension through the modulation of estrogen receptor.

Conclusion

The present work concludes, *Paederia foetida* whole plant extract has an antihypertensive action in Amphetamine-induced hypertensive rats which is evident by a considerable decrease in blood pressure. Amphetamine induces decreasing sodium level that can be countered by *Paederia foetida* whole plant extract, whereas sodium & potassium level remains to be same in all the four groups of rats. According to the OECD (Organization of economic co-operation and development 425 guidelines) *Paederia foetida*, whole plant extract was safe for administration at 200 and 400 mg/kg. Among the two dosages tested for *Paederia foetida*, whole plant extract 400 mg/kg body weight exhibited the highest considerable antihypertensive effect. Geniposide, a natural moiety found in this plant, is having antihypertensive activity. Docking study was carried out on different PDB ID to find out the potency of the compound as an antihypertensive. Geniposide, an antihypertensive agent present in this plant, is used for in silico study to define its potential as an antihypertensive agent. The ligand structure was docked with different PDB IDs (3OLL, 3OLS, 5DX3, 5DXE & 6PIT). The docking studies revlealed the PDB ID: 3OLS to be most suitable in terms of its comparison with the internal ligand, Estradiol. Further molecular dynamics simulation study was carried out to evaluate the binding between Geniposide and estrogen receptor. The findings indicate that Geniposide forms stable interactions with key amino acid, GLU305 of β-ligand binding domain of estrogen receptor, suggesting its potential as an activator of estrogen receptor. Thus, Geniposide can be a useful compound for the treatment of hypertension through the modulation of estrogen receptor.

Abbreviations

PDB ID	Protein data bank identifier
SDF	Structure data file
WNK	With-no-lysine kinase
Pdb2gmx	GROMACS program
CHARMM27	Chemistry at Harvard Molecular Mechanics
TIP3P	Transferable intermolecular mechanics potential with 3 points
LINCS	Linear constraint solver
PME	Particle Mesh Ewald
ADME	Absorption distribution metabolism elimination
CPK	Corey–Pauling–Koltun
NPT isobaric	Isothermal ensemble
NVT	Constant volume ensemble
PDBQT	Protein data bank, partial charge (Q), and atom type (T) format
OPLS	Optimized potentials for liquid simulations
BBB	Blood–brain barrier
GI	Gastrointestinal
MR	Molar refractivity
PAINS	Pan Assay Interfering Substances
TPSA	Topological polar surface area
VMD	Visual Molecular Dynamics
ps	Pico seconds
ns	Nanoseconds
K	Kelvin
MD	Molecular dynamics
RMSD	Root-mean-square deviation
RMSF	Root-mean-square fluctuation
nm	Nano meter
p.o.	Orally
Å	Amstrong

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-024-00633-1.

Additional file 1: Figure S1: Systolic blood pressure of Amphetamine-induced rat along with *Paederia foetida* whole plant extract (200, 400 mg/kg/day, p.o., for 4 weeks) and Hydralazine as a standard drug; **Figure S2:** Graphical representation of Sodium level in serum of Amphetamine-induced rat along with *Paederia foetida* whole plant extract (200, 400 mg/kg/day, p.o., for 4 weeks) and Hydralazine as a standard drug; **Figure S3:** Graphical representation of Potassium level in serum of Amphetamine-induced rat along with *Paederia foetida* whole plant extract (200, 400 mg/kg/day, p.o., for 4 weeks) and Hydralazine as a standard drug; **Figure S4:** 2D Molecular interaction of: a) Geniposide & b) Estradiol with 3OLL; **Figure S5:** 2D Molecular interaction of: a) Geniposide & b) Estradiol with 3OLS; **Figure S6:** 2D Molecular interaction of: a) Geniposide & b) Estradiol with 5DX3; **Figure S7:** 2D Molecular interaction of: a) Geniposide & b) Estradiol with 5DXE; **Figure S8:** 2D Molecular interaction of: a) Geniposide & b) Estradiol with 6PIT; **Figure S9:** Toxicity radar chart of a) Geniposide & b) Estradiol.

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Author contributions
Conceptualization, supervision, formal analysis, writing, review and editing were done by Suddhasattya Dey and Arijit Mondal; Ravi Rawat, Dibya Lochan Mohanty, Deeparani Urolagin, Ameeduzzafar Zafar, Padma Charan Bahera, and Chanchal Koley helped in investigation; Naresh Kumar Rangra, Volkan Eyupoglu, Ravi Rawat, Arijit Mondal, Suddhasattya Dey, and Anjan Mondal were involved in software, methodology, and validation. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Institutional Animal Ethical Committee of Aditya Bangalore Institute of Pharmacy Education and Research Bangalore, Karnataka, India (approval number: 64/1611/CPCSEA). Female Wistar rats (Wistar strain) weighing between 150 and 200 g were obtained from local vendor, Bangalore, Karnataka. Animals were housed into five groups under standard laboratory conditions, i.e., 25 °C ± 1 °C/45–55% RH and 12/12 h light and dark conditions in the animal house of Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, Karnataka, India. The rats are kept with free access to food (Hindustan Lever, India).

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no known competing interests or personal relationship that could have appeared to influence the work report in this paper.

Author details
[1]Department of Pharmaceutical Technology, Bengal College of Pharmaceutical Technology, Ward No. 8, Cinema Hall By Pass Road, Notunpally, Dubrajpur, West Bengal 731123, India. [2]Department of Pharmacy, IQ City Institute of Pharmaceutical Scienes, Durgapur, West Bengal 713206, India. [3]Department of Pharmaceutical Technology, School of Health Sciences and Technology, UPES, Dehradun 248007, India. [4]Department of Pharmaceutical Technology, ISF College of Pharmacy, Ghal-Kalan, G.T. Road, Moga, Punjab 142001, India. [5]Department of Pharmaceutical Technology, RR College of Pharmacy, Dasarahalli Main Rd, Raja Reddy Layout, Medaralli, Chikkabanavara, Hesaraghatta, Karnataka 560090, India. [6]Department of Pharmaceutics, School of Pharmacy, Anurag University, Hyderabad 500088, India. [7]Department of Pharmaceutics, College of Pharmacy, Jouf University, 72341 Sakaka, Al-Jouf, Saudi Arabia. [8]Department of Chemistry, Cankırı Karatekin University, 18100 Cankırı, Turkey.

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