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Research Article

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Polyphenols from Prunus armeniaca.L as Promising Anticancer (Cervical Cancer): In silico studies and in vivo safety assessment

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Abstract

Some anogenital tract malignancies have high-risk human papillomavirus (HPV) infections as their etiological cause. Although many HPV preventative vaccines have been licensed, there is still a need for medication that targets the infection and its carcinogenic effects. One of the important elements in cell immortalization and tumor development in HPV-positive cells has been identified as the viral oncoprotein E6. The cellular ubiquitin ligase E6AP interacts with E6, which can facilitate the degradation of the tumor suppressor protein p53. One of the best ways to prevent the maintenance and growth of infected cells is to block the creation of the E6-E6AP complex. The present study aims to determine the ability of polyphenols identified in Prunus armeniaca.L, to target the HPV16 virus by virtual high-throughput screening and molecular docking, and to evaluate the safety of this plant in vivo. In silico, the PDB: 4GIZ structure of E6HPV16 was prepared as a target by Discovery Studio 2021. Virtual screening of 47 polyphenols was performed by the iGEMDOCK program, followed by an evaluation of potential inhibitors based on docking affinities obtained from the The SYBYL-X Surflex-Dock module v2.0, 21. In vivo toxicity studies of Prunus armeniaca. L aqueous extract was also conducted in Wistar rats. Of all the polyphenols investigated in this study, the compounds 3-pCoumaroylquinic, 5-pCoumaroyloquinic, Epicatechin, and Dimethoxyflavone were predicted to have the highest binding affinity for E6HPV16, also revealed several interactions with the E6 binding site area. A study on acute in vivo toxicity of Prunus armeniaca .L aqueous extract was conducted and didn't produce any harmful effects. Moreover, Epicatechin, a dimethoxyflavone from Prunus armeniaca.L, 3-pCoumaroylquinic, 5-pCoumaroylquinic, and 5-pCoumaroyloquinic were chosen as possible E6HPV16 inhibitors for novel medication development.

Keywords: Cervical cancer; E6-E6AP; Docking; Prunus armeniaca.L; ADMET properties; toxicity

Introduction

Human papillomaviruses (HPV) are small, double-stranded, circular DNA viruses of the HPV family. They are part of the Papillomaviridae family. There are more than 200 different HPV genotypes that infect mucosal and epithelial cells of human skin, a subset of which are tumorigenic[1] .The viral genome consists of seven genes classified as early genes (E1, E2, E3, E4, E5, E6, and E7), which control viral transcription and genome replication, and two Late genes (L1 and L2) encode the structural proteins involved in capsid formation [2]. HR-HPV16 is the major etiological factor in most cases of cervical cancer [3]. HR-HPV-induced carcinogenesis depends primarily on the expression of HPV-en-

coded E6 and E7 oncogenes, which are synergistically involved in the malignant conversion of infected cells due to their ability to degrade p53 and Rb, respectively [4]. The HPV E6 oncoprotein cooperates with several cellular proteins activating various oncogenic pathways that lead to blockage of apoptosis, changes in the transcriptional machinery, interference with cell-cell interactions, and cell immortalization [5]. HR E6 oncoproteins are involved in the regulation of p53 gene transactivation and can abolish the transcriptional transactivation activity of p53 [6]. HR E6 oncoproteins are also able to interact with p300/CBP co-activators to control p53-dependent gene regulation [7].

HPV E6 proteins are rather small (about 150 amino acids), are cysteine-rich, and share a common architecture consisting of two zinc-binding domains (E6N and E6C) with a conserved fold that is connected by a helical linker [8]. The E6 amino-terminal zinc-binding domain and the carboxy-terminal zinc-binding domain have a globally conserved fold in the crystal [9]. The two zinc domains, along with an alpha helix tube that connects them, form a deep pocket in which the LXXLL peptide makes close contact [10]. LxxLL is a 20 amino acid peptide in E6AP, which results in recruitment and polyubiquitination of p53. The LxxLL peptide isolated from E6AP is sufficient to make E6 susceptible to interaction with p53 [11]. The central pocket of E6 binds to the LxxLL motif of the ubiquitin ligase E6AP, resulting in a conformational change of the E6 HR proteins allowing the creation of a complex with p53. In this ternary complex, called E6/E6AP/p53 [12, 13].

To date, three prophylactic vaccines have been validated and effectively used to control persistent viral infections and HPV-associated cervical lesions. As a result, the management of cervical cancer and precancerous lesions is still limited to the use of chemotherapeutic agents and/or surgical and ablative techniques to remove developed tumors. Both of these treatments are invasive, non-specific, and tend to be expensive, making their availability limited for millions of patients, usually in developing countries [14]. Therefore, the development of affordable drug therapies targeting the onco-virus is essential for the specific treatment of HPV-related diseases and improved management of cervical cancer and precancerous lesions.

The population of Deraa Tafilalt region has great experience in using medicinal plants to fight against various diseases. Indeed, UNESCO has declared this region a biosphere reserve Medicinal and aromatic plants are a real source of phytocompounds that have been used in the treatment of various diseases caused by microorganisms. Through numerous pharmacological tests, plants and their derived components have proven their antibiotic, antimitotic, and antiviral activity [15, 16] .

With this in mind, we wanted to determine if medicinal plants are used by the population. We used molecular docking techniques to evaluate the efficacy of Prunus armeniaca. L compounds against E6HPV16. In addition, a safety assessment through acute and subchronic in vivo studies was performed.

Materials and methods

In silico studies

Data set

A series of 47 phenolic compounds that were extracted in the plant Prunus armeniaca. L, their molecular structures were obtained from the database pubchem these molecules were considered for the study of in-silico [17].

Preparation of the target

The 3D X-ray crystal structure of Crystal structure of full-length human papillomavirus oncoprotein E6 in complex with LXXLL peptide of ubiquitin ligase E6AP (PDB ID: 4GIZ), was obtained from the Protein Data Bank[18]. They were prepared by Discovery Studio 2020 by removing water molecules and adding polar hydrogen [19]. The structure of 4GIZ was extracted in PDB format and saved for inclusion as a target in docking.

Virtual screening

High-speed virtual screening was performed using the iGEMDOCK (Generic Evolution Method for Docking) program [20]. The in silico screening of 47 phytocompounds was performed using the PDB code of the targets (PDB ID: 4GIZ) chain "C", the screening score, which is based on total energy calculations (total energy = VdW + HBond + electrostatic), was calculated using iGEMDOCK v2.1.11.

The standard parameters used for screening; population size, generations, and number of solutions were set at 300, 70 and 2, respectively. Energy-based results were analyzed and 12 potential inhibitors were selected based on stability for further detailed analyses.

Prediction of ADMET

To develop a drug, it is necessary to go through several steps, starting with target identification and ending with ADMET prediction. Early detection of these properties is therefore very necessary to decrease the cost and duration of the drug development process. To define the passage of this drug in the organism, an evaluation of pharmacokinetic and ADMET parameters (adsorption, distribution, metabolism, excretion and toxicity) was performed, in this perspective, 16 phenolic compounds that were selected in the screnning step were evaluated to determine these pharmacokinetic parameters in silico using ADMETSAR[21] . and pkCSM, in order to prevent the failure of these compounds in clinical trials and increase their chances of reaching the stage of drug candidates in the future [22].

Molecular docking

Molecular docking is a technique for predicting receptor-ligand interaction in drug discovery. Using this method, several studies have proposed certain molecules as good candidates for treating several pathologies.

The ligands 3-pCoumaroylquinic, 5-pCoumaroyloquinic, and Epicatechin, Dimethoxyflavone, were docked to the 'C' chain of the unit cell of the crystal structure (PDB: 4GIZ). The SYBYL-X Surflex-Dock module v2.0, 21 were used to generate bioactive binding positions of ligands in the E6HPV16 active site [23]. After completing docking, the ligand pose gave the minimum binding energy. Discovery studio and PyMOL were used to visualize the results. The results of the molecules that showed an interesting docking score were analyzed and their positioning inside the active site was compared [19, 24]. The type of interactions established by each molecule inside the active site was also compared.

In vivo toxicity studies

Plant material

The Prunus armeniaca L. leaves were collected from the Tafilalet region (semi-arid area) of Morocco in May 2022, and air-dried at 40 °C. The plant was taxonomically identified and authenticated, and a voucher specimen was deposited at the herbarium of the Faculty of Sciences and Techniques of Errachidia under the number PA22.

Preparation of the aqueous extract

The aqueous extract of the plant material was prepared according to the most traditional method used in Morocco (decoction): 1 g of powdered leaves mixed with 100 ml distilled water, was boiled for 10 min and then cooled for 20 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2 mm, St Quentin en Yvelines, France) to remove particulate matter. Finally, the filtration of the extract was lyophilized in a lyophilizator (LABCONCO, G.BOYER, materiel de laboratoire, Casablanca). Doses administered for acute toxicity were 1, 2 and g freeze-dried aqueous extract per kg body weight [25] .

Experimental animals

Healthy albino adult male rats (Wistar strain) with a weight ranged between 150 and 210 g were housed under standard environmental conditions (23 ± 1 oC with 55 ± 5% humidity and a 12 h/12 h light/dark cycle) and maintained with free access to water and ad libitum standard laboratory diet.

Acute toxicity study

In the current study, safety assessment was carried as described previously, with slight modifications and in accordance with the organization for Economic Cooperation and Development (OECD) [26, 27]. Briefly, healthy female rats were treated orally by a limit dose of 2000 mg/kg of Prunus armeniaca aqueous extract (PAAE). Firstly, one overnight fasted rat was treated with PAAE (2000 mg/kg body weight) then mortality and signs of toxicity were monitored hourly follow-up for three hours after the dosing and then periodically throughout 48 h. If the first rat dosed survived sequentially other rats were treated, and finally observation was made for 14 days. If three or more rats survived, the LD50 was predicted to be above 2000 mg/kg. All applicable guidelines for the care and use of animals were followed (FSTE/2015) [28].

Sub-chronic toxicity study

Twelve female Wistar rats were divided into two groups and their weights were recorded. The first group received 2000 mg/kg body weight of PAAE orally once daily for 28 days. The control group received distilled water. During the experimental period, toxic manifestations and mortality were observed. After 28 days of treatment all animals were fasted overnight, body weight was recorded and all rats were anaesthetized, and blood samples were collected for biochemical analyses and blood pressure parameters measurement. Additionally, heart, lungs, liver, spleen and kidneys were dissected and weighed at the end of the experiment.

Relative organ weight

After sacrificed by cervical dislocation, organs were weighed and their index in relation to body weight was calculated as described in: ROW (g) = weight of organ/bodyweight of rats on the day of sacrifice × 100% [29].

Effect on liver enzymes and chlorides

Serum alanine aminotransferase (ALT) activity was estimated using the modified kinetic method of Srivastava et al., using a kit supplied by SGM, Italia, according to the instructions of the supplier. Serum aspartate aminotransferase (AST) activity was assessed using the modified kinetic method of Schumann and Klauke, using a kit supplied by SGM, Italia, according to the instructions of the supplier. Serum chlorides were estimated using SGM, Italia, according to the instructions of the supplier [30].

Blood pressure measurement

For estimation of blood pressure parameters, systolic blood pressure (SBP), mean blood pressure (MBP), and heart rate (HR) were measured using a tail-cuff and a computer-assisted monitoring device (Harvard, Boyer, Casablanca, Morocco) as described previously [31]. In brief, Systolic blood pressure (SBP), mean blood pressure (MBP) and heart rate (HR) were measured directly using pulse tracing while diastolic blood pressure (DBP) was calculated from SBP and MBP using the formula: DBP= (3MBP-SBP)/2.

Glycemia determination

Blood glucose levels were determined by the glucose oxidase method using a reflective glucometer (Contour™ TS) from Bayer Diabetes Care (ref).

Statistical analysis

Data were expressed as mean ± SEM. Statistical differences among the means studied were assessed by two-way ANOVA with GraphPad Prism 6 software. Differences were considered to be significant when $p<0.05$. Whereas, data obtained from the relative organs weights were analyzed using unpaired sample T-test at a 95% confidence interval, with Welch's correction.

Results

In silico studies

Virtual screening

In the present study 47 phytocompounds of Prunus armeniaca. L

was screened against the E6 HPV16 protein. In this step, 16 molecules were selected based on their binding affinity to E6 HPV16 protein for further evaluation (Table 3).

Prediction of ADMET

Lipinski's Rule of Five (Ro5) prediction results of these compounds are shown in Table 1. The results of logP values and molecular weights of all designed compounds except 3-O-Caffeoylquinic compound, indicating that they have reasonable absorption and are moderately soluble in water, were in perfect agreement with the most important rules of drug similarity. In addition, the HBA of Quercetin-3- O - glucoside, Quercetin-3-O_6-acetyl-glucoside, Kaempferol-3- O - rutinoside,Proanthocyanidin A2,Procyanidin B1,Procyanidin B2, Quercetin-3-O_6-acetyl-glucoside, Kaempferol-3- O - rutinoside. HBD of Chlorogenic acid, Cis-5-Caffeoylquinic acid, Neochlorogenic acid the number of rotational bonds of Kaempferol-3- O - rutinoside indicated that these eleven compounds do not conform to Lipnski's rules (Table 1).

Table 1: Molecular properties for predicting the drug sensitivity of the potential inhibitors.

Compound name	MW	Log P	HB Acceptor	HB donor	Rotating bonds
Procyanidine B1	578.526	2.995	12	10	3
Épicatéchine	290.271	1.5461	6	5	1
Chlorogenic acid	364,39	-0.4	8	6	4
Cis-5-Caffeoylquinic acid	354.311	-0.4	9	6	5
Neochlorogenic acid	354.311	-0.6459	8	6	4
5-p-Coumaroylquinic acid	338.312	-0.3515	8	5	5
5-oCaffeoylquinic acid	354.311	-0.6459	9	6	5
3-p-Coumaroylquinic acid	338.312	-0.3515	8	5	5
3-OCaffeoylquinic	678.599	0.4	8	4	4
Kaempférol-3- O - rutinoside	594.5	-0.9	15	9	6
Acide 4-O-caféoylquinique	354.31	-0.6459	8	6	4
Caféoyl-glucoside	342.3	-1.5459	9	6	4
caryophyllene	204.357	4.7252	0	0	0
Quercetin	302.23	1.5	7	5	1
Rosmarinic	360.3	2.4	8	5	7
Dimethoxyflavone	282.29	2.2	4	0	3

The results of the predictions of absorption, distribution, metabolism, excretion and toxicity are presented in Table 2. For the pkCSM predictive model, the compounds 3-pCoumaroylquinic, 5-pCoumaroyloquinic, and Epicatechin, caryophyllene, Quercetin, Dimethoxyflavone show good absorption and excellent distribution properties, this could be envisioned as permeable molecules with low distribution in the brain. They also have good clearance and no inhibition of the hERG system or AMES toxicity. The cytochrome P450 subtypes CYP2D6 and CYP3A4 indicate that 3-pCoumaroylquinic, 5-pCoumaroyloquinic, and Epicatechin, caryophyllene, Quercetin, Dimethoxyflavone, could not be substrates or inhibitors for the two major subtypes, and therefore probably could not be metabolized, resulting in a low risk of drug interactions. We suggest that they are promising inhibitors and were selected for the docking.

Table 2: ADMET in silico prediction of identified E6HPV16 inhibitors

	Épicatéchine	5-p-Coumaroylquinic acid	3-p-Coumaroylquinic acid	Dimethoxyflavone
Absorption and Distribution				
Blood-brain barrier (logBB)	-1.054	-1.16	-1.16	0.429
Intestinal absorption (human)	68.829	43.925	43.925	97.629
Caco-2 permeability	-0.283	-0.656	-0.656	1.33
P-glycoprotein substrate	Yes	yes	yes	yes
P-glycoprotein inhibitor	no	no	no	no
Metabolism				
Substrat CYP2D6	no	no	no	no

Substrat CYP3A4	no	no	no	yes
Inhibiteur CYP2D6	no	no	no	no
Inhibiteur CYP3A4	no	no	no	yes
Excretion and Toxicity				
Clearence	0.183	0.453	0.453	0.561
hERG I inhibitor	no	no	no	no
Carcinogens	no	no	no	no
AMES toxicity	no	no	no	no
Hepotoxicity	no	no	no	no

Molecular docking

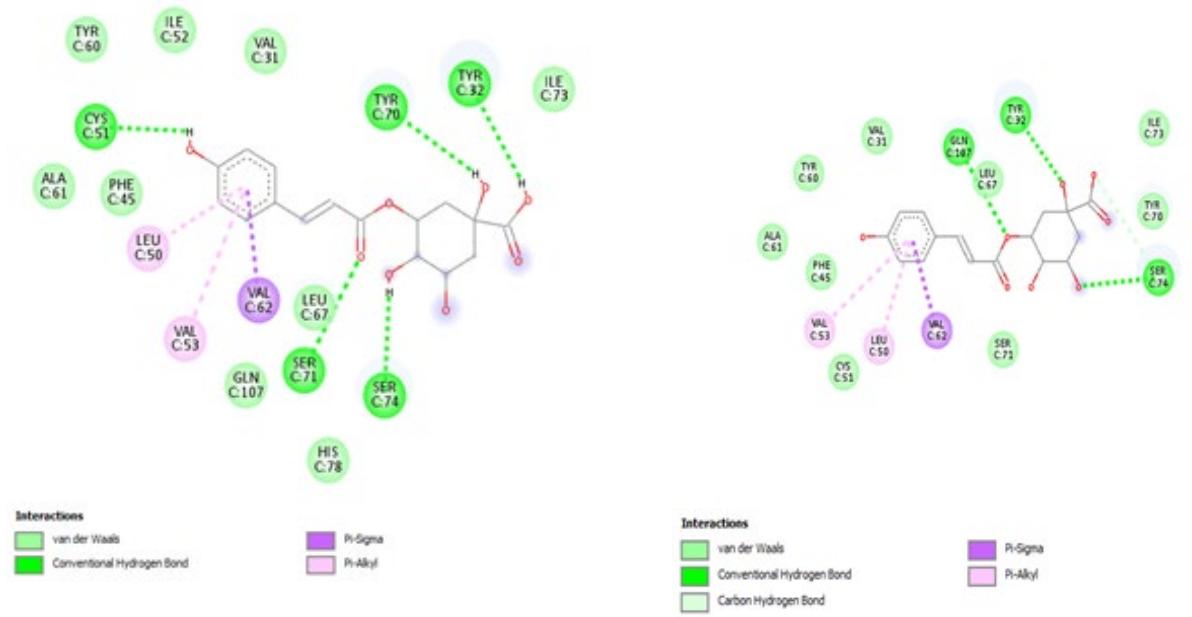
The 4 compounds with the highest affinity selected after the virtual screening approach were docked to the HPV16 E6 active site. Based on their score values, these molecules were scored and ranked. Drugs with docking scores between -6.2 and -6.7 were considered interesting compounds that can be proposed as potential candidates to inhibit the HPV16 E6 receptor The 4 candidates with the highest docking score are presented in Table 3. The positioning of these 4 molecules with HPV16 E6 was visualized.

Table 3: Docking results showing binding affinities of phytocompounds and established hydrogen interactions with amino acids.

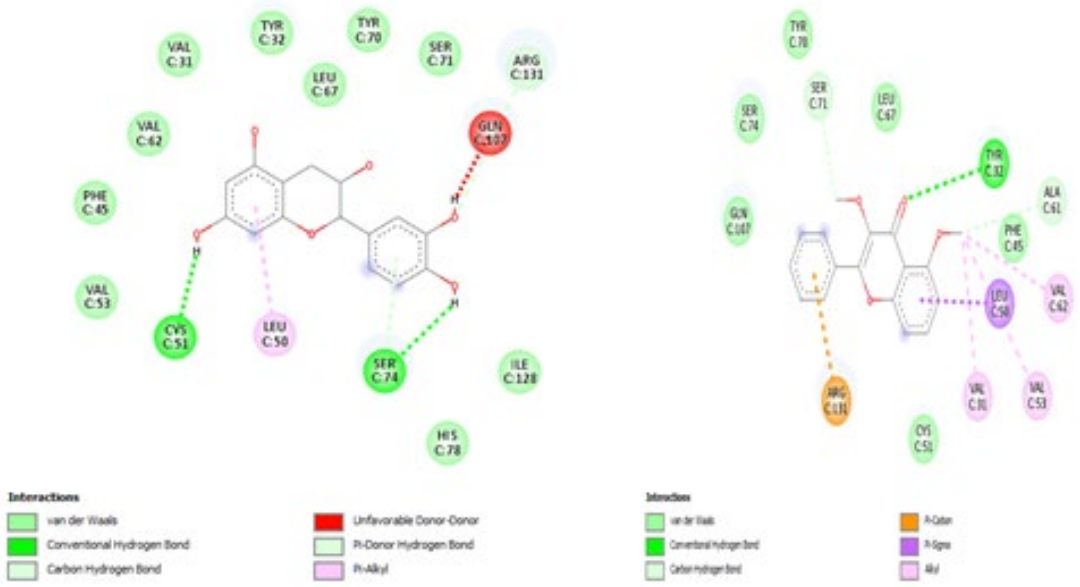
Compound name	PubChem CID	Hydrogen bonds	Total Energy	Binding affinity
3-p-Coumaroylquinic acid	9945785	Ser(74), Ser(71), Cys(51), Tyr(70), Tyr(32)	-70.7856	-6.7
5-pCoumaroyloquinic	90478782	Gln(107), Tyr(32), Ser(74)	-73.7024	-6.9
Épicatéchine	72276	Cys(51), Ser(74),	-66.5173	-6.2
Dimethoxyflavone	88881	Tyr(32)	-66.6708	-6.6

Visualization and analysis of results

we observed that all four candidate compounds share common interactions and are involved in H-bond type interactions with Ser(74), Cys(51), Tyr(32) residues, which is consistent with recent studies [32]. Figure 1.



3-p-Coumaroylquinic acid 5-pCoumaroyloquinic



Épicatéchine Dimethoxyflavone

Figure 1: Types of interactions between the E6HPV16 active site and selected potent Prunus armeniaca L inhibitors.

In vivo toxicity studies

Acute toxicity

Prunus armeniaca aqueous extract (PAAE) was given orally once at a dose of 2000 mg/kg b.w without producing any toxicity symptoms or fatalities. Additionally, there was no significant difference in body weight between treated and control rats. However, it was shown that this herb's LD50 value was greater than 2000 mg/kg. Sub-chronic toxicity

Sub-chronic toxicity

Daily oral administration of PAAE at a dose of 2000 mg/kg b.w for 28 days did not cause any toxicity signs or death. When compared to the control group, also the body weight of the treated rats did not increase significantly, in addition to relative organs weight (ROW) of heart, Kidneys, Spleen. In contrast, the ROW of Lungs ($p<0.05$) and Liver ($p<0.0001$) were significantly increased in PAAE treated group (Table 4). Table 5 represents the biochemical and blood pressure parameters of the treated and control groups. The results showed that PAAE at a high dose of 2000 mg/kg b.w did not significantly alter systolic, mean and diastolic blood pressure, heart rate, chlorides, plasma AST and ALT levels by compared to the untreated group. However, blood glucose level ($p<0.05$) slightly decreased significantly after 28 days of dosing.

Table 4: Body weight and relative organ weights of rats treated orally with PAAE.

groups	Day	Body weight (g)	Heart (%)	Lungs (%)	Kidneys (%)	Liver (%)	Spleen (%)
Control	D0	186.2 ± 27.71	0.34 ± 0.02	0.62 ± 0.06	0.59 ± 0.05	2.77 ± 0.19	0.22 ± 0.04
	D28	201.4 ± 18.82					
PA 2 g/kg	D0	166.66 ± 3.5	0.41 ± 0.03	0.92 ± 0.08*	0.7 ± 0.03	3.85 ± 0.08****	0.23 ± 0.02
		181.66 ± 6.72					

Values expressed as mean ± SEM, n=6. ****p<0.0001.

Table 5: Biochemical values and blood pressure parameters of rats treated with PAAE for 28 days.

Biological parameter	Day	Control	PAAE 2 g/Kg
Glycemia (mg/dL)	D0	96.6±10.06	102.00±5.69
	D28	97.80±8.04	76.33±1.60*

SBP	D0	130.25±8.99	127.83±1.88
	D28	132.5±14.93	119.22±1.81
MBP	D0	110.75±12.28	96.83±2.00
	D28	115.75±15.52	92.5±4.41
DBP	D0	101±14.74	81.33±2.68
	D28	107.38±16.11	77.33±6.17
HR	D0	293.25±19.65	298.8±15.79
	D28	302.25±3.77	297.6±12.77
AST	D0	20.97±5.75	28.15±2.07
	D28	26.19±8.61	37.68±3.27
ALT	D0	30.96±5.26	23.54±1..93
	D28	26.31±6.58	18.26±1.43
Chlorides	D0	76.70±13.70	102.15±13.13
	D28	84.39±14.81	109.50±12.18

Values expressed as mean ± SEM, n=6. *p<0.05.

Discussion

HPV is the agent responsible for the development of cervical cancer in women. It is among the most severe and deadly malignancies in women that we find, the formation of complexes between the HPV oncoprotein, E6 with the cellular ubiquitin ligase E6AP allows to trigger the implementation of modulation, such as attenuation of telomere shortening, immortalization, host cell differentiation, control of cellular pathways, regulation of growth factors, degradation and inactivation of tumor suppressors, disruption of DNA repair efficiency and apoptosis and facilitate cell transformation and hTERT gene increment, Therefore, suppression of the creation of the E6-E6AP complex is one of the essential strategies to inhibit the survival and proliferation of infected cells [33]. Although surgery, radiation therapy, hormone therapy, combination chemotherapy and immunotherapy as well as the validation of several prophylactic vaccines, there is a lack of effective prognosis, therefore there is no effective treatment for persistent HPV infection. On the other hand Medicinal plants have received much attention to treat many diseases, studies have explored the role of plant metabolites in cancer treatment, and these metabolites may be one of the solutions to target drugs against HPV induced cancers. In our present study, we screened 47 polyphenols from P. Armeniaca L for their efficacy in targeting E6HPV16 using an in silico approach. In a previous study, the authors revealed the beneficial action of Prunus Armeniaca L seed extract used in liver cancer therapy[34] . Based on their total binding energy, this step allowed us to select 16 molecules. These molecules were selected as the compounds with the highest stability and affinity (binding energy higher than -50.8508 and lower than -93.6087kcal/mol). According to Lipinski, a drug with promising potential is one that meets the five adopted rules, namely, Log P partition coefficient less than 5, weight (MW) less than 500 Da, HBA number<10, HBD number<5, and rotational bonds less than 10. Among the 15 molecules, 3-pCoumaroylquinic, 5-pCoumaroyloquinic, and Epicatechin, Dimethoxyflavone, have chemical and physical properties that allow them to be used as active drugs, as they comply with Lipinski's rules. On the other hand, the other molecules indicated that these 12 compounds do not comply with the Lipinski rules revealing violations towards OR5. The 4 compounds selected by the virtual screening approach and showed the best pharmacokinetic profiles were docked to the active site of E6HPV16, using Autodock vina and MGL Tools programs, the results of the current study showed a higher docking score (-6.2and -6.9) of the selected polyphenols and revealed several interactions with the active site of E6HPV16. Compound toxicology testing is a crucial step in the development of pharmaceutical products. Preclinical toxicity tests reveal adverse or toxic effects specific to the species and the dose of an experimental product [35]. Acute toxicity tests provide preliminary information on the toxic nature after administration of a single dose of a test substance to determine the dose that will cause serious toxicological effects that occurs either immediately or at a short-term. They also serve to provide information on doses that should be used in subsequent studies, such that the estimation of LD50 is critical in carrying out toxicological investigations on chemicals, including plant extracts [36, 37]. In the present study, female Wistar rats received a single oral dose of 2000 mg/kg b.w of PAAE, and signs of toxicity and mortality were observed for 14 days. During this period, the results revealed no deaths, changes in animal behavior or signs of toxicity developed in both the control and treated group. Therefore, the LD50 of PAAE was above 2000 mg/kg b.w. As per UN Classification, any substance which has oral LD50 of more than 2000 mg/kg b.w is considered as low hazard potential and categorized as UN 6.1 PG III [38]. Sub-chronic studies aim to assess adverse effects of continuous or repeated exposure of plant extracts or compounds over for a specified period up to the expected lifespan of the test species. They provide information on general characteristics of the toxicity, the toxicity to specific target organs, responses to toxic metabolites formed in the organism, delayed respon [39]. To achieve this goal a sub-chronic study was established at the same dose of the extract during 28 days of daily treatment.

Similarly, the sub-chronic oral administration of PAAE caused no mortality and no behavioral changes in the animals throughout the 28-day study period. Thus, no change in body weight during the acute (14 days) and sub-chronic (28 days) toxicity study was observed. Likewise, changes in body weight have been used as an indicator for detecting adverse effects of drugs and chemicals [40]. Significant changes in relative organ weights are considered a relative indicator of potential toxicity of the substance[41]. The results of this study revealed that the relative organs weights (ROW) of the heart, kidneys, spleen in the treated groups were not significantly different. In contrast, the ROW of Lung and Liver were significantly increased in PAAE treated (2000mg/kg b.w) group when compared to the control group, that may reflect hypertrophy of this plant at this high dose used. Assessment of biochemical parameters is critical in assessing organ function, most especially kidney and liver. They have significant roles as a marker because of their response to clinical signs and symptoms produced by toxicants. Evaluation of hepatic and renal function is of prime importance to assess the toxic properties of extracts and drugs [42]. Non-significant differences were seen in biochemical parameters (AST, ALT, and chloride values) except mean values of serum glucose showed significant decrease compared with control. The result is consistent with previous studies which showed that polyphenol-rich Prunus armeniaca leaf extract has an ant-diabetic effect by inhibiting α-glucosidase and α-amylase [43]. However, further studies need to be conducted on the effect of PAAE on the liver and lungs. Blood pressure parameters had no impact after 28 days from treatment, indicating its safety on the cardiovascular system despite the high dose used. De plus, la dose utilisée dans l'activité antihypertensive précédemment démontrée était de 100 mg/kg b.w, soit 20 fois inférieure à la dose utilisée dans les études de toxicité [43].

Conclusions

By applying the in silico approach to detect protein-ligand interactions, we identified four potential candidates with the highest binding energy scores that would be potential inhibitors of HR E6 oncoproteins, presumably without significant side effects. Furthermore, this study highlights the value of using the virtual screening approach as a time- and cost-efficient strategy to identify chemicals with potential biological effects as well as Administration of an aqueous extract of Prunus armeniaca (PAAE) did not result in mortality or clinically significant changes in the biological parameters tested, with the exception of hypoglycemia, and the relative organ weights (ROW) of the lungs and liver were significantly increased during 28 days of PAAE administration.

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