INVESTIGATION OF POTENTIAL ACTIVITIES OF *PEGANUM HARMALA* SEEDS: *IN SILICO* AND *IN VITRO* ANALYSES

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Seeds of *Peganum harmala* L. have been traditionally used in Algerian medicine. This study investigates whether the antioxidant, antihemolytic, and anti-inflammatory activities of *Peganum harmala* extracts (PHE) are attributed to polyphenolic compounds, which are abundant in methanol, chloroform, and ethyl acetate extracts.

Extraction and fractionation of polyphenols involved solvents with different polarities, resulting in a crude extract (CrE), a chloroform extract (CHE), and an ethyl acetate extract (EAE). The antioxidant potential of CrE and its fractions was assessed using the ferrothiocyanate (FTC) and thiobarbituric acid (TBA) assays. The inhibition of mice erythrocyte hemolysis was evaluated for methanol, chloroform, and ethyl acetate extracts in the presence of the oxidant (AAPH). PMA-induced mouse ear edema was used as an *in vivo* model for inflammation.

The FTC assay demonstrated the strong antioxidant effect of CrE (87.64 ± 0.003%). EAE showed potent antioxidant activity with low MDA absorption levels. Assessment of antihemolytic effects against AAPH-induced oxidative hemolysis revealed significant protective effects of CrE and EAE, with EAE showing the most pronounced effect. In the *in vivo* model, CrE (100 mg/kg) exhibited substantial anti-inflammatory activity, inhibiting the ear edema. Computational analyses using molecular docking simulations showed that chlorogenic acid, hesperetin, and rutin have promising potential as inhibitors of COX-2 protein, which is a key component in inflammatory pathways.

This study highlights the potent antioxidant properties of *P. harmala*, particularly in CrE, and its anti-inflammatory effects. The bioactive compounds, such as chlorogenic acid, hesperetin, and rutin, exhibit potential as anti-inflammatory agents. *P. harmala* could be a valuable natural source for potential medical applications, suggesting the need for further exploration in medical treatments.

Keywords: *Peganum harmala*, antioxidant, hemolytic, anti-inflammatory, ear edema, molecular docking

INTRODUCTION

Medicinal plants are known to have many active compounds, which have been used to treat various diseases.[1] Among these compounds, polyphenols and flavonoids stand out as secondary metabolites with antioxidant properties. These compounds play an important role in neutralizing free radicals, reducing the risk of heart disease, cancer and premature aging. Moreover, polyphenols are known to inhibit the generation of free radicals, thus preventing diseases such as cancer, infection, inflammation and immune system dysfunction.[2]

Several studies highlighted the potential antioxidant and health benefits of phenolic compounds. Erythrocytes are a valuable model for investigating oxidative pathways and protective networks. The erythrocyte membrane, which is rich in polyunsaturated fatty acids, is particularly sensitive to peroxidation damage caused by free radicals. Such damage to the erythrocyte membrane by free radicals can lead to bleeding.[3,4]

Inflammation is a wonderful defense mechanism activated by dangerous substances. It involves increased permeability of blood vessel walls, resulting in more immune cells at the site of injury, leading to edema and eventual tissue repair. Several researchers have documented the link between oxidative stress and inflammatory response. In this context, various reactive compounds, such as superoxide radicals, produced by macrophages during inflammation can contribute to various diseases, such as autoimmune diseases, allergies, hepatitis and hepatitis injury.[6,7]

Peganum harmala L. (Zygophyllaceae) is known for its phytotherapeutic properties and is one of the commonly used annual plants in traditional medicine for treating diseases associated with inflammation response.[8] Studies in the literature have shown that *P. harmala* has many beneficial properties, including antihistaminic and immunomodulatory properties, and that it is effective in the treatment of leukemia.[9,10] Phytochemical screening of *P. harmala* has revealed the presence of various substances, such as flavonoids, alkaloids, steroids, saponins, anthraquinones, polysaccharides and amino acids in its fruits, leaves, stems and flowers.[11]

Our objective in this study was to evaluate the anti-haemolytic and anti-inflammatory properties of *P. harmala* extracts. To our knowledge, the seeds of this plant have never been examined for these specific properties.

EXPERIMENTAL

Plant materials

The seeds of *Peganum harmala* L. were harvested in July from Batna, Algeria, and identified by Professor Laouar Houcine from the Department of Ecology and Vegetal Biology at Setif 1 University, Algeria. Subsequently, the seeds were dried in the dark at room temperature and then powdered.

Animals

Healthy male Swiss albino mice weighing 20 to 30 g were used in this study and housed in cages of 6–8 mice each. Food and water were available *ad libitum* and a 12-h light/dark cycle was maintained at 25 °C. All animal experimental procedures were approved by the Animal Ethics Committee of Setif 1 University, Algeria. The experiments were conducted in accordance with the guidelines and protocols of the International Animal Experimentation System and adhered to the principles of the 2008 Declaration of Helsinki, with a particular focus on minimizing suffering.

Extraction of phenolic compounds

Phenolic compounds were extracted from *Peganum harmala* L. using different chemicals with different polarities, as described by Markham (1982).[12] Harmal powder was dissolved in methanol at a concentration of 1:10 w/v and stirred constantly overnight at 4 °C. The resulting precipitate was then filtered to obtain a preliminary extract, and the solvent was extracted at a temperature below 45 °C under reduced pressure (Rotavapor: Germany, Büchi461), resulting in a methanol extract (called "CrE"). Liquid-liquid extraction procedures were used through the series: hexane for lipid extraction, chloroform for aglycone flavonoid extraction, and ethyl acetate for glycoside flavonoid extraction. The resulting percolate was heated to give chloroform and ethyl acetate extracts (ChE and EaE, respectively). All were stored at -20 °C for further analysis.

Quantification of flavonoid compounds

Total flavonoid content was determined using the aluminum chloride method.[13] In this method, 1 mL of each quercetin extract or standard solution was mixed with 1 mL of 2% $AlCl_3$. After 10 min of incubation, the absorbance was measured at 430 nm against the blank. Results were expressed as quercetin equivalents per gram of dried plant extract (mg QE/g DW), calculated using a quercetin calibration curve.

Quantification of total phenolic content

The total phenolic content was determined using the Folin Ciocalteu reagent.[14] Each extract (100 µL) was mixed with 500 µL of diluted (10-fold) Folin Ciocalteu reagent, followed by the addition of 400 µL of a 7.5% Na_2CO_3 solution after a 4-minute interval. The mixture was then vigorously shaken and incubated in the dark at room temperature for 1 hour, after which the absorbance was measured at 760 nm. The total polyphenol content of the extracts was calculated using a gallic acid standard curve and expressed as milligrams of gallic acid equivalent (GAE) per gram of dried plant extract.

In vitro antioxidant activity

Ferric thiocyanate (FTC) assay

The inhibitory effect on lipid peroxidation was assessed using the ferric thiocyanate assay. A mixture containing 155 µL of linoleic acid and 158 µL of Tween 20 in phosphate buffer (pH 7, 0.02 M) was prepared, to which 500 µL of the extract was added. This mixture was then incubated at 40 °C in the dark. After incubation, 0.1 mL of ammonium thiocyanate (30%), 4.7 mL of ethanol (75%), and 0.1 mL of ferrous chloride (20 mM) were added to 100 µL of the incubated mixture. The absorbance was measured at 500 nm to evaluate the inhibitory effect on lipid peroxidation.[15]

Thiobarbituric acid (TBA) assay

The TBA assay was conducted on the final day of the FTC method, following the procedure outlined by Tunalier *et al*.[16] 1 mL aliquot of the solution mixture from the FTC method was combined with 2 mL of trichloroacetic acid and 2 mL of thiobarbituric acid. The resulting solution was heated in a boiling water bath for 10 minutes. After cooling, the solution was centrifuged for 20 minutes at 3000 rpm, and the absorbance of the supernatant was measured at 532 nm.

Preparation of cell blood suspensions: hemolysis assay

Blood samples were obtained from Swiss albino mice and centrifuged at 3000×g for 10 minutes to separate the erythrocytes. The erythrocytes were then suspended in phosphate-buffered saline (PBS) to create a 2% suspension. To induce free radical oxidation in the erythrocytes, 2% hematocrit suspensions were incubated with or without AAPH 300 mM (2,2,-azobis (2-amidinopropane) dihydrochloride), which generates free peroxyl radicals. In this assay, 80 µL of the hematocrit suspension was combined with 20 µL of the extract (0.1 mg/mL), and the mixture was incubated for 15 minutes at 37 °C. Subsequently, 136 µL of AAPH solution (300 mM) was added to induce hemolysis of the erythrocytes. The kinetics of erythrocyte resistance to oxidative degradation was determined at 37 °C by measuring the continuous changes in density at 620 nm.[17] The percentage inhibition of hemolysis was calculated using a specific equation:

% Hemolysis inhibition = absorbance of control - absorbance of sample / absorbance of control ×100 (1)

PMA-induced mouse ear edema

To measure the anti-inflammatory effect, we used the PMA-induced mice ear oedema assay. Ear edema was induced by PMA on mouse ears according to the modified method of Garrido.[18] The control group received PMA alone; while two other groups were treated by 100 mg/kg of methanol (CrE+PMA) and 10 mg/kg of Diclofenac (Diclofenac +PMA). Briefly, 4 µg/ear PMA in 20 µL DMSO was applied topically to the inner surface of the right ear of each mouse; the left ear received the vehicle (20 µL DMSO). CrE and Diclofenac were administered 1 h before PMA application. After 6 h of PMA application, mice were euthanized by cervical dislocation, and a 6 mm diameter round was removed with a metal punch and weighed. Ear edema weight was calculated by the equation:

Edema weight = the weight of right disc – the weight of the left disc (2)

Molecular docking study

To further understand the anti-inflammatory effects of phenolic compounds extracted from *P. harmala* seeds against Cyclooxygenase-2 (COX-2), a protein associated to inflammation, a molecular docking research was conducted. In a study by Moazeni *et al*.,[19] five phenolic compounds, including p-coumaric acid, rutin, catechin, hesperetin, and chloregenic acid, were identified in *P. harmala* seed extracts. These compounds were used to carry out molecular docking in the presence of the reference molecule Diclofenac. The 3D structures of the specified phenolic compounds and reference molecule were obtained using PubChem.

The 3D crystal structure of COX2 (PDB ID: 1CX2) in complex with SC-558 was downloaded from the PDB. The protein data file was imported into the molecular docking program AutoDock 25 in the pdb format.[20] First, using Biovia Software, the co-crystallized ligand was removed, further trimming the protein. The protein was then processed, including the removal of water molecules, extra-chains or heteroatoms, the addition of hydrogen, the calculation of charges (Kollman charges), and conversion to a pdbqt file. A 40 x 40 x 460 point grid with 0.5-centered spacing was used. The coordinates for the grid box were (x = 13.085, y = 52.413, and z = 70.43). The protein macromolecules were maintained rigid throughout the docking simulation, and the docking parameters were established using the Lamarckian Genetic Algorithm 4.2.[20] We only used 50 genetic algorithms and did not change any other docking parameters. Based on the highest binding affinities, the best protein-ligand conformations were found using the AutoDock4.2 scoring program. The BIOVIA Discovery Studio Visualizer 4.1 was used for post-docking analysis. Complexes of docked protein-ligand and co-crystallized proteins were employed to verify the docking method. Using BIOVIA Discovery Studio Visualizer 4.1, the docked complexes were aligned and superimposed over the reference co-crystallized protein complexes to calculate comparative root-mean-square deviation (RMSD) values.

Statistical analysis

All experiments were expressed as mean ± standard deviation. The differences between the results were determined by ANOVA (one-way analysis of variance), followed by Tukey's multiple comparison test. All results were analyzed using the GraphPad Prism, p value ≤ 0.05 was considered as significant.

RESULTS AND DISCUSSION

Total polyphenol and flavonoid contents

The total phenolic content rankings in *P. harmala* extracts, determined by the Folin-Ciocalteu method and expressed as mg GAE/g DW, followed a descending order: ChE exhibited the highest content, followed by EaE, and finally CrE. Similarly, the rankings for flavonoid content, assessed by the aluminum chloride method and expressed as mg QE/g DW, mirrored this order: ChE had the highest flavonoid content, followed by EaE, and CrE (see Fig. 1).

These results clearly indicate that the chloroform extract had the highest levels of total polyphenols (74.67 mg GAE/g DW) and flavonoids (21.32 mg QE/g DW).

Antilipid-peroxidation activity using FTC and TBA assays

The extraction yields showed that the crude extract CrE registered a yield of 1.65 ± 0.27%.

Phenolic compounds and flavonoids in CrE were 27.91 ± 0.98 µg GAE/mg extract and 7.39 ± 0.68 µg QE/mg extract, respectively. In contrast, chloroform (CHE) and ethyl acetate (EAE) extracts contained phenolic compounds and flavonoids in amounts of 66.29 ± 1.58 and 13.88 ± 0.13 (µg GAE/mg extract) respectively, and 58.1 ± 2.87 and 12.18 ± 0.08 (µg QE/mg extract), respectively. The researchers found that methanol was the most effective solvent for the extraction of various polyphenols due to its high polarity.[21] Polyphenols are a class of water-soluble molecules, and FTC and TBA assays were used to evaluate the antioxidant properties of the extracted materials by examining lipid peroxidation.

Lipid peroxidation is a chain reaction involving the oxidative degradation of lipids, resulting in tissue damage through the action of free radicals. The inhibition of peroxidation by the extracts indicates their antioxidant properties. The higher percentage of lipid peroxidation in each phase indicates the presence of more internal antioxidants responsible for scavenging free radicals, especially hydroxyl radicals, and delaying hydroperoxide formation.[22]

The inhibition percentage of CrE (87.64±0.003%) was higher than that of EAE (80.52±0.04%) and CHE (74.67±0.007%), respectively, while the inhibition percentage of CrE showed no significant changes ($p>0.05$) compared to vitamin C (87.87±0.004%) and BHT (87.68±0.012%) (Fig. 2).

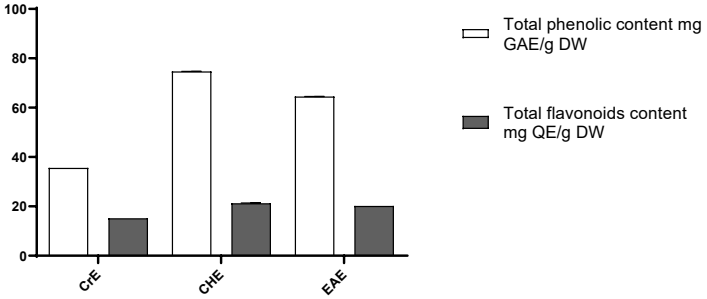

Figure 1: Total polyphenol and flavonoid contents in *P. harmala* extracts

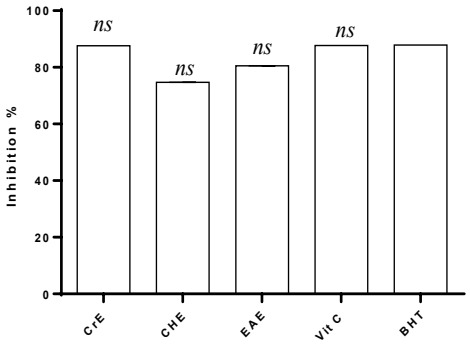

Figure 2: Percentage inhibition and absorbance values of linoleic acid oxidation by PHSE after 3 days (ns: $p > 0.05$); measured by FTC method (mean ± SD)

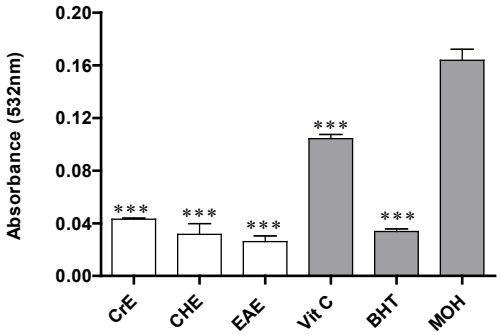

Figure 3: Absorbance at 532 nm of PHSE by the TBA method, compared with BHT and Vit C (mean ± SD; $n = 3$)

The thiobarbituric acid reactive substance (TBARS) assay is employed to measure malondialdehyde (MDA) products, which are end-products resulting from lipid peroxidation. In this assay, MDA reacts with thiobarbituric acid (TBA) to form a red chromogenic complex that is measured at 532 nm. The results of the TBA assays are depicted in Figure 3. The absorbance of EAE (0.026±0.004) was significantly lower ($P < 0.001$) than that of Vit C and BHT (0.104±0.003 and 0.033±0.002, respectively), indicating that EAE exhibited a significantly higher inhibition of lipid peroxidation, followed by CHE and CrE. This high peroxidation scavenging effect may be attributed to the substantial phenolic or flavonoid contents or antioxidant activity present in the extracts, which can inhibit peroxide formation and other biological molecules.[23]

Antioxidant effect on red blood cells (hemolysis test)

Erythrocytes are the most abundant cells in the circulatory system, with membranes rich in polyunsaturated fatty acids, oxygen, and hemoglobin, making them vulnerable to oxidative stress and hemolysis.[24] The production of free radicals by the decomposition of AAPH molecules in the presence of transition metals induces lipid and protein peroxidation, which can damage erythrocyte membranes, leading to hemolysis and cell death.[25] This assay aimed to evaluate whether *P. harmala* fractions could prevent the oxidation of erythrocyte membranes. The study investigated the scavenging effect of peroxyl radicals induced by hemolysis in the presence of *P. harmala* extracts. Erythrocytes (2%) were incubated at 37 °C in phosphate buffer, and when AAPH was added to the erythrocyte suspension, hemolysis was induced. The *P. harmala* extract exhibited a differential pattern of anti-hemolytic activity. CrE and EAE showed potent anti-hemolytic action at 0.1 mg/mL, with CrE (56.65% RBC membrane stabilization), exhibiting the maximum inhibition of hemolysis, followed by EAE. However, CHE induced erythrocyte lysis at the same concentration, with a hemolysis percentage of 65.92% (Fig. 4).

The protection against hemolysis indicates the presence of endogenous antioxidants in red blood cells (RBCs), such as catalase, superoxide dismutase, α-tocopherol, and glutathione, which can effectively scavenge free radicals to protect against hemolysis.[26] The hemolytic action of CHE could be attributed to the cytotoxic effects of some saponins present in this plant, which form complexes with biomolecules, such as phospholipids, proteins, and sterols, thereby modifying the permeability of the erythrocyte membrane.[27] However, the protective activity of CrE and CHE may be due to the presence of polyphenols, particularly flavonoids, which are associated with the anti-hemolytic action. Polyphenols are known to attack the oxidation of the erythrocyte membrane by binding near their tryptophan residues.[3] The protective ability of flavonoid derivatives depends on their chemical structure, with hydroxyl (OH) substitutions and a C2=C3 bond in the C ring, along with their liposolubility, increasing their anti-hemolytic capacity. They act as antioxidant compounds that incorporate into the membrane and quench free radicals before they can attack the membrane, thereby preventing erythrocyte membrane oxidation.[25] The anti-hemolytic activity of the extracts may be attributed to their inhibitory effect on enzymes involved in the production of chemical mediators of inflammation, as well as on the metabolism of arachidonic acid.[28] Some polyphenols can incorporate into membrane proteins, leading to a change in their conformation.[29] Others may bind to phospholipase A2 (PLA2) through hydrophobic interactions with three amino acids from the enzyme's active site.[30]

PMA-induced ear edema in mice

PMA application was employed to induce an acute inflammatory response, acting by activating protein kinase C and releasing various inflammatory mediators, such as cytokines, proteases, and NADPH oxidases, leading to the production of histamine and contributing to cell damage.

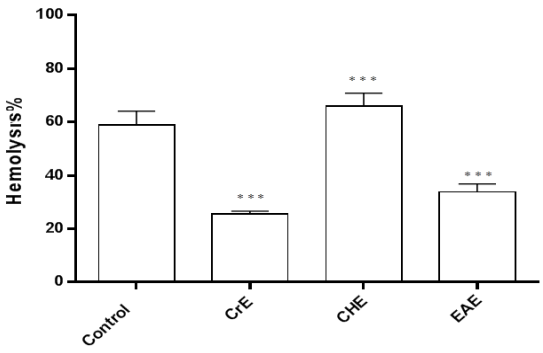

Figure 4: Effects of *P. harmala* extracts on AAPH-induced hemolysis (comparisons are made with respect to the control group, ***: $p \leq 0.001$, (n=3))

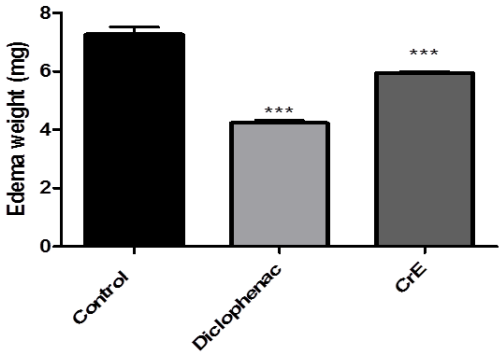

Figure 5: Inhibition of PMA induced ear edema by CrE and diclophenac applied topically before PMA application (each point represents the mean ± SEM (n=7-8); $p \leq 0.01$; $p \leq 0.001$ vs. control group)

This acute inflammatory reaction persists for 6 hours and diminishes after 24 hours. Excessive application of PMA can result in inflammatory disorders, such as epidermal cell hyperplasia and inflammatory cell influx.[6,31]

The results depicted in Figure 5 show that the edema weight of the control group (7.5 ± 0.08 mg) was reduced in the diclofenac group (a standard anti-inflammatory drug) to 4.1 ± 0.06 mg and in the methanol group to 6.3 ± 0.2 mg. The CrE exhibited an inflammatory inhibition percentage of 19.54%. The inhibitory activity of the methanolic extract was significantly ($p \leq 0.001$) greater compared to the diclofenac group.

The anti-inflammatory potential of the extract may be attributed to the presence of polyphenolic compounds, particularly flavonoids, such as cynaroside, and phenolic acids, such as hydrocaffeic and protocatechuic acids, which are abundant secondary metabolites in *P. harmala* extract. These polyphenols have shown higher antioxidant and antiproliferative activities.[32,7] Additionally, compounds like tannins, flavonoids, and saponins have the ability to inhibit pain sensitivity by interfering with enzymes involved in inflammation, particularly the synthesis of prostaglandins and arachidonic acid metabolism. Tannins could reduce the inflammatory response by inhibiting iNOS in macrophages and possessing free radical scavenging properties, while saponins can reduce inflammation by inhibiting NO.[33]

The anti-inflammatory effects of methanol extracts are correlated with their levels of phenolic and flavonoid compounds, as well as their antioxidant properties. These findings are consistent with a study by Edziri and colleagues,[34] which demonstrated that methanol extracts from both *Marrubium alysson* and *P. harmala* effectively inhibit carrageenan-induced paw edema and exhibit strong antioxidant activity, characterized by high total phenolic content. Another study showed that the ethyl acetate extract of *P. harmala* displayed significant inhibition of 70.3% at a dose of 200 mg/kg against carrageenan-induced paw edema. Preliminary analysis of the extract indicated the presence of alkaloids and flavonoids.[35]

Molecular docking

The standard (Diclofenac) and five phenolic compounds from *Peganum harmala* were docked to the COX-2 active site (PDB ID: 1CX2). The ligands were classified in Table 1 based on the binding energy (kcal/mol) that they had with the protein. The binding energies of the complexes, as well as the interactions between COX-2 and ligands, were used to examine the data. According to the findings, chloregenic acid (-12.63 kcal/mol) exhibited the strongest binding affinity for COX2 protein, followed by hesperetin (-11.42 kcal/mol), rutin (-11.28 kcal/mol), and catechin (-11.23 kcal/mol). The standard molecule Diclofenac had a low binding affinity (-9.64 kcal·mol^{-1}). The inhibition constants of the docked targeted protein receptors with the investigated drugs are shown in Table 1.

The interaction patterns of the targeted protein COX2 and the compounds were analyzed and visualized using BIOVIA Discovery Studio Visualizer 4.1 (Table 1 and Fig. 6). As shown in Table 1, chlorogenic acid interacts with COX2 protein catalytic site with a strong affinity by forming H-bonds with residues Gln192, Tyr385, and Ser530, which showed distances between 2.10 to 3.01 A°.

Table 1
Ligand binding scores and interactions with COX-2

Molecules	Docking score, kcal/mol	KI	H-bonds	Hydrophobic
p-Coumaric acid	-6.78	10.68 uM	Ala527	Met522
Rutin	-11.28	5.41 nM	**Tyr385, Gln192, Tyr355, Arg120**	Ala527, Val89, Ser353, Ser530, **Val523**
Catechin	-11.23	5.88 nM	**Gln192**, Gly526	**Val523**, Ala527, Met522 Leu352, Ala516
Hesperetin	-11.42	4.26 nM	**Tyr385, Arg120, Tyr355**	Met522, Ala527, Leu352, Val116, Leu359, Val349
Chloregenic acid	-12.63	549.61 pM	**Tyr385, Ser530, Gln192**	Met522, Ala516, Leu352, **Val523**
Diclofenac	-9.64	86.13 nM	-	Met522, Leu352, **Trp387**, **Val523**, Val349, Ala527, Leu531

Note: The amino acids in bold correspond to the active site amino acids

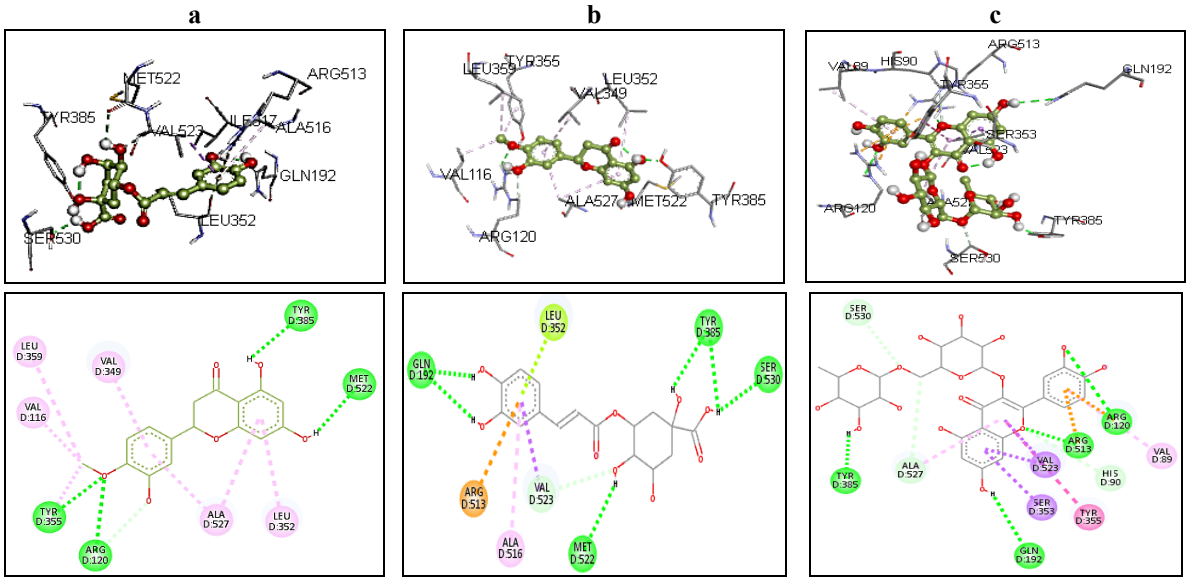

Figure 6: Visualization of ligand-receptor interactions in 3D (above) and 2D (below); a: chlorogenic acid, b: hesperitin, c: rutin

H-bonds were also shown between Tyr385, Tyr 355 and Arg120 with hesperetin (2.11 to 2.55 A°) and Tyr385, Arg120, Tyr355, Gln192 with rutin (1.75 to 5.37). Chlorogenic acid displayed hydrophobic interactions with COX-2 protein, such as the amino acid residues Ala516 Val523 and Leu352. Hesperetin and rutin were found to form hydrophobic interaction with COX-2 protein, such as the amino acid residues of Met522, Leu352, Ala527, Val116, Leu395, Val349 and Ala527, Val523, Val89 respectively.

ADMET analysis

The drug-likeness and toxicological properties of chemical ligands are important determining factors for their use as medications. To investigate the suitability of phenolic compounds from *P. harmala* seeds as a prospective target for therapeutic development, *in silico* methods were utilized to estimate ADMET parameters. Four of the five phenolic compounds included in the table do not violate Lipinski's rule of five for oral availability, indicating that the compounds have drug-like molecular nature (Table 2), with the exception of rutin, which has MW>500 g/mole, nHA (> 5) and Nhd > 16. These compounds, except rutin, are in the optimal range of the physiochemical space, and might therefore be regarded as lead compounds.

The pharmacokinetic parameters revealed that the p-Coumaric acid is highly absorbed through the intestine as the reference compound (diclofenac), unlike rutin and chlorogenic acid, which are considered as poorly absorbed. Also, all compounds, except rutin and chloregenic acid, showed limited Blood-Brain Barrier (BBB) penetration with values <-1. The CNS permeability values obtained demonstrated that all substances are unable to reach the central nervous system, except the reference molecule, which has a CNS value of -1.97 (logPS > 2). The steady state of distribution (VDss) is estimated to be low when log VDss0.15 and high when log VDss>0.45, and it was found that the p-Coumaric acid VDss is low compared to diclofenac, while the other compounds showed higher VDss.

For drug metabolism, it is important to establish whether the medicine can inhibit cytochrome P450, which is a crucial detoxifying enzyme in the body, mostly found in the liver and oxidizes xenobiotics to encourage their excretion. The compounds under study do not inhibit the isoforms, except diclofenac, which inhibits CYP2C9 isoform.

The range of the total clearance of the substances under study reported as log (mL/min/kg), was -0.369 to 0.662. Moreover, all of the compounds considered in this work are unlikely to behave as an OCT2 substrate. Characteristics, such as mutagenicity, tumorigenicity, reproductive effect, irritating effect, AMES toxicity, hepatotoxicity, and hERG I inhibitors, were estimated for toxicity prediction. All the compounds provided no toxicity risk, except the reference molecule diclofenac, which excretes reproductive toxicity.

Table 2
Physicochemical properties, drug-likeness, metabolism, absorption, excretion, and toxicity of phenolic compounds of *Peganum harmala* seeds and diclofenac

	p-Coumaric acid	Rutin	Catechin	Hesperetin	Chloregenic acid	Diclofenac
Physicochemical properties						
MW (mg/mol)	164.050	610.150	290.080	302.080	354.100	295.020
nHA	3	16	6	6	9	3
nHD	2	10	5	3	6	2
Drug-likeness						
Lipinski's rule	Yes	No	Yes	Yes	Yes	Yes
Bioavailability score	0.85	0.17	0.55	0.55	0.11	0.85
Absorption						
Water solubility (log mol/L)	-2.378	-2.892	-3.117	-3.047	-2.449	-3.863
Caco2 permeability (log Papp in 10-6 cm/s)	1.21	-0.949	-0.283	0.294	-0.84	1.379
Intestinal absorption (human) %	93.494	23.446	68.829	70.277	36.377	91.923
Skin Permeability (logkp)	-2.715	-2.735	-2.735	-2.737	-2.737	-2.724
P-glycoprotein substrate	No	Yes	Yes	Yes	Yes	Yes
P-glycoprotein I inhibitor	No	No	No	No	No	No
P-glycoprotein II inhibitor	No	No	No	No	No	No
Distribution						
VDss (human) (logl/kg)	-1.151	1.663	1.027	0.746	0.581	-1.605
BBB permeability (logBB)	-0.225	-1.899	-1.054	-0.719	-1.407	0.236
CNS permeability (log PS)	-2.418	-5.178	-3.298	-2.976	-3.856	-1.97
Metabolism						
CYP1A2 inhibitor	No	No	No	Yes	No	No
CYP2C19 inhibitor	No	No	No	Yes	No	No
CYP2C9 inhibitor	No	No	No	Yes	No	Yes
CYP2D6 inhibitor	No	No	No	Yes	No	No
CYP3A4 inhibitor	No	No	No	Yes	No	No
Excretion						
Total clearance (log mg/kg/day)	0.662	-0.369	0.183	0.044	0.307	0.291
Renal OCT2 substrate	No	No	No	No	No	No
Toxicity						
Mutagenicity	No	No	No	No	No	No
Tumorigenicity	No	No	No	No	No	No
Reproductive Effect	No	No	No	No	No	Yes
Irritating Effect	No	No	No	No	No	No
AMES toxicity	No	No	No	No	No	No
Hepatotoxicity	No	No	No	No	No	No
hERG I inhibitors	No	No	No	No	No	No

CONCLUSION

Medicinal plants have been utilized worldwide to treat various ailments, including inflammation, heart diseases, and cancer. The results of this study suggest that *P. harmala* seeds possess anti-lipid peroxidation and antioxidant activities, which may contribute to the prevention of several diseases. Moreover, CrE demonstrates a significant *in vivo* inhibitory effect on inflammation induced by PMA. Comparing these results with those obtained in hemolysis, it can be suggested that the possible mechanism of inhibition involves a dual action of the extracts, with CrE and EAE inhibiting hemolysis while CHE induces hemolysis. These activities could be attributed to the nature of polyphenolic compounds as well as the synergistic effect of all fraction compounds. The molecular docking study revealed the effectiveness of rutin, catechin, hesperetin, and chlorogenic acid, which exhibited

a high binding affinity for the target proteins COX2, compared to the reference molecule diclofenac. Additionally, ADMET analysis suggested that phenolic compounds extracted from *P. harmala* might be a promising medicine that is safer than diclofenac. In conclusion, this study suggests that *P. harmala* seeds could be used as a complementary source of medicine to treat various diseases when the antioxidant, anti-inflammatory, and anti-hemolysis systems are impaired.

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