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Antipyretic, Anticonvulsant, And Hepatoprotective Properties of Aqueous Extract of *Atriplex Halimus* Leaves: In Silico And In Vivo Studies

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ABSTRACT

The main objective of using natural healing remedies is to mitigate the adverse effects associated with synthetic pharmaceuticals. This study aimed to assess the potential of a water-based extract derived from the leaves of *Atriplex halimus* in alleviating fevers, preventing seizures, and safeguarding the liver in mice using *in vivo* models and *in silico* methods. The investigation includes the protection of mice's livers from damage induced by paracetamol and monitoring liver markers such as ALP, ALT, AST, and total bilirubin in the blood, as well as assessing superoxide dismutase (SOD), MDA, catalase (CAT), and GSH levels in liver tissue.

Administration of plant extracts and silymarin to rats significantly mitigated the adverse effects of paracetamol on serum and tissue markers. Histological analysis of the liver revealed a notable impact of the extracts in reducing the severity of liver damage, with hepatoprotective efficacy comparable to that of silymarin.

Significant antipyretic effects were also observed within two hours of administering various doses of the extract, and these effects persisted throughout the experiment. Furthermore, the plant extract was found to be efficient to protect from seizure 33.34% to 83.33% of the mice treated with isoniazid and diazepam.

Finally, the pharmaceutical properties, including pharmacokinetics and toxicity of the main compounds found in *A. halimus* extract, were examined by ADMET simulations, and molecular docking studies were employed to investigate interactions between these compounds and drug targets.

Keywords: *Atriplex halimus* extract, fever-reducing, liver-protective, anti-seizure, rodents

Introduction

A febrile seizure is a neurological condition arising from a peripheral infection. In response, the immune system triggers an inflammatory reaction, leading to a fever and an increase in the body's core temperature. Rising temperatures cause heightened neural excitability, leading to convulsions. At present, fever management involves the use of different analgesics, including NSAIDs, which may cause several adverse effects, such as gastrointestinal bleeding, renal

impairment[1], and severe liver damage. Therefore, the ongoing pursuit of novel treatments with fewer side effects and improved effectiveness persists [2]. Herbal medicines are commonly perceived as having reduced toxicity and fewer negative effects in comparison to synthetic treatments. Multiple medicinal plants have shown potential as innovative and reliable therapeutic options[3]. According to Stringer [4], more than 50 plants have exhibited anticonvulsant effects. On the other hand, Xiao et al. observed that Chinese traditional medicine utilizes 23 botanicals for epilepsy treatment, but none of them have been transformed into a standardized drug for seizures[5]. Numerous significant pharmaceuticals, such as artemisinin, vincristine, vinblastine, quinine, and morphine, are derived from bioactive chemicals isolated from medicinal plants. These medications are used for the treatment of pain, inflammation, convulsions, cancer, malaria, and various other ailments[6–10]. The *Atriplex* genus (Amaranthaceae) constitutes herbaceous halophytes, including about 260 species distributed worldwide, especially in the arid and semi-arid regions of Europe, Asia, Africa, Australia, and North America[11]. Recent studies have shown that some species have high nutritional value and protein content and can be used as cereal grains. For example, seeds of A. hortensis[12]. Chemical analysis of certain Atriplex species has identified a range of compounds from several chemical families, including phenolics[13], triterpenes, sterols[14], phytoecdysteroids[15,16], and triterpene saponins[13,14,17]. From a biological perspective, several species have been documented to possess anti-inflammatory[18], antioxidant[19], anticholinesterase[13], antidiabetic[20], antimicrobial[21], hepatoprotective[22], immunomodulatory, analgesic, antipyretic, and cytotoxic properties[23,24].

Atriplex halimus, also known as *A.halimus*, is a rich source of vitamins A, C, and D[20]. It also includes tannins, flavonoids, saponins, alkaloids, and resins, and has a salt chloride content of up to 10%[25]. A. halimus is a species found in both non-saline and saline habitats, in locations ranging from sub-humid to desert in South Europe, East Mediterranean, and North Africa, including Algeria[26,27]. This plant is acknowledged in traditional medicine for its exceptional properties in treating diseases and is utilized as a medicinal plant in traditional pharmacopeia [28]. In Algeria, the foliage and upper parts of this plant are used for alleviating gastrointestinal ailments. Additionally, they possess medicinal properties such as healing, antispasmodic, antidiabetic, and anti-inflammatory effects. Despite the long-standing usage of A. halimus for pain management and inflammatory conditions. Nevertheless, there have been no comprehensive empirical investigations carried out to ascertain its impact on fever, convulsions, and hepatoprotective properties. Hence, it was imperative to examine the antipyretic, anticonvulsant, and hepatoprotective effects of the aqueous extract of *A.halimus* leaves (AEAL) in mouse animal models.

Materials and methods

Plant collection and identification

The *Atriplex halimus* specimens were gathered in May 2021 at Es-Senia, a seaside location in the province of Oran, situated in western Algeria. The coordinates of the location are 35º38´N, 00º36´W, with an elevation of 92 meters. The plant has been identified by the Botany Department of the Higher National Agronomic School of Algiers, Algeria. A voucher specimen has been

stored at the Giffen Herbarium in the Food Hygiene and Quality Assurance Research Laboratory (HASAQ), located in Algiers, Algeria, for future reference

Animals

Healthy Swiss albino mice of either sex (female for acute toxicity and male for the main study) aged 6–8 weeks and having a weight range of 28–36 g were used for the experiment. The mice were housed in polypropylene cages (6 mice per cage) under standard environmental conditions and with a 12-hour dark/light cycle. (26 ± 3 °C, 65 ± 1% relative humidity, and a light control room). They were fed standard pellet feed and water ad libitum. The experiments with animals were performed as per the legislation for protecting animals used for scientific purposes.

Drugs and chemicals

Paracetamol, isoniazid, diazepam, and saline water (NaCl, 0.9 %) were obtained from Saidal (Algeria). Silymarin was purchased from XETION laboratories (Algeria) All other chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich.

Aqueous extract preparation

In this experiment, 100 grams of powdered *A.halimus* were soaked in 1 liter of distilled water for 48 hours. The macerate has been subjected to two rounds of filtering, initially employing cotton wool and then passing it through the Whatman filter paper (Number 1). The filtrate was separated from its solvent by subjecting it to heat under reduced pressure in a rotating evaporator at a temperature of 40°C. Afterward, the gathered product was subjected to lyophilization using a lyophilizer for 12 hours. The resultant product was subsequently stored in a refrigerator at a temperature of 4°C for future use.

Acute toxicity test

The study methodology was conducted following the guidelines set forth by the Organization for Economic Cooperation and Development (OECD) in 2000, instruction 423[53]. The experimental cohort, consisting of 5 female mice, received an oral dose of AEAL at a concentration of 5000 mg/kg. The control group of mice, consisting of 5 female individuals, were administered distilled water. Signs of toxicity and death were observed at 1, 2, 4, and 6 hours following oral administration of an AEAL, and subsequently once daily for 14 days. Measurements of animal weights, mortality, and observable alterations were documented.

Antipyretic activity

The evaluation of the ability to decrease body temperature was conducted by inducing hyperthermia in mice using yeast, following the experimental protocol outlined by Kumar et al.[54]..Fever was produced by administering a subcutaneous injection of 10 mL/kg of a 20% yeast suspension (yeast group). The chosen animals underwent a period of fasting during the night. The animals' initial rectal temperature was measured using a digital thermometer. Animals that had a rise in rectal temperature of more than 2°C after receiving a subcutaneous injection for 18 hours were chosen for the study on the effectiveness of fever reduction, following 18 hours of yeast infusion. The oral administration of AEAL was conducted at dosages ranging from 250 to 500 mg/kg. The experimental group was administered PCM (50 mg/kg, oral) as the reference medicine, whereas the control group was given normal saline (10 mL/kg) as a placebo. The rectal

temperature was taken at 0, 1, 2, 3, and 6 hours. The administration of all medications was done via the oral route.

Anticonvulsant activity

The animals were categorized into V-groups, with each group consisting of 6 mice (n = 6 mice in each group).

Group I - Normal control

Group II - Negative control Isoniazid 300mg/kg i.p.

Group III - Standard diazepam 10 mg/kg + Isoniazid 300mg/kg i.p

Group IV - Low dose 250mg/kg p.o + Isoniazid 300mg/kg i.p.

Group V - High dose 500mg/kg p.o + Isoniazid 300mg/kg i.p. Animals The INH Model is a pharmacologically induced technique used to assess the anti-epileptic efficacy of a medication. The treatment and standard groups were statistically compared to the vehicle groups. Both oral delivery of vehicles and extracts, as well as subcutaneous (s.c.) administration of conventional medicines, were employed. On the fourteenth day, extracts were given before the initiation of seizures. Seizure is provoked in groups II, III, IV, and V with the subcutaneous dose of 300 mg/kg of isoniazid in mice. The condition of the animal was documented, along with the percentage of protection it exhibited according to (Vogel & Vogel)[55].

In vivo hepatoprotective activity

The 30 animals were divided into five groups, with six mice in each group.

G-I served as normal control and received 0.5% (CMC) carboxy received for 7 days.

GII served as PCM control, and received paracetamol (2 g/kg) for seven days.

G-III served as reference control, and received silymarin (200 mg/kg) once daily for 7 days along with PCM 2 g/kg).

G-IV and G-V were treated with AEAL (250 mg/kg and 500 mg/kg respectively) once daily for 7 days along with PCM (2 g/kg).

All the test drugs and PCM were administered orally by suspending them in 0.5% CMC solution. After 24 h of the last dose of PCM, the blood was collected from the retro plexus, after blood collection, the animals were sacrificed by cervical dislocation, and the liver was dissected out and used for biochemical studies and histological examination. The hepatoprotective activity of the extract was evaluated by calculating the percentage protection[56] :

$$\%\, protecton = \frac{a-b}{a-c}\ 100$$

(a) the mean value of the marker produced by hepatotoxin; (b) the mean value of the marker produced by toxin plus test material, and (c) the mean value produced by the vehicle control.

Biochemical analysis

The spectrophotometric method was used to evaluate the activity levels of liver enzymes, specifically aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin. Commercially available diagnostic kits were used for this purpose.

Oxidative stress markers

The liver tissue from the sacrificed experimental animals was washed and homogenized in a 1:10 ratio of weight to volume in ice-cold 50 mmol/L Tris buffer at a pH of 7.4. The contents underwent centrifugation at a force of 10,000 times the acceleration due to gravity for a duration of 20 minutes at a temperature of 4 degrees Celsius. The resulting liquid above the sediment was then examined for the presence of superoxide dismutase (SOD)[57], catalase (CAT)[58], and glutathione (GSH)[59]. Lipid peroxidation by-product malondialdehyde (MDA)[60], was measured in the form of thiobarbituric acid reactive substance (TBARS) by Ohkawa et al[61].

Histopathological studies in the liver

After conserving the liver tissue in a 10% formalin solution for 24 hours, the tissue was placed in paraffin and cut into 5-millimeter-thick sections using a rotary microtome. The sections were subsequently stained with hematoxylin-eosin (H&E) dye to detect hepatic damage and examined under a microscope to observe histological alterations in the liver[62].

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant

In silico studies

Drug likeness and ADMET profile

In silico studies have become indispensable for assessing the potential of new therapeutic agents. These computational tools efficiently predict the bioavailability of molecules, offering quick insights[63]. The main chemical species found in the extract of *Atriplex hallimus*[64] underwent screening for drug-likeness and ADMET properties using SwissADME[65], admetSAR[49], and ProTox-II[66] web servers.

Key bioavailability parameters such as octanol-water partition coefficient (log P), total polar surface area (TPSA), number of hydrogen bond acceptors (HBA), and number of hydrogen bond donors (HBD) were evaluated. Assessment of drug-likeness adhered to established criteria like Lipinski's rule[67] and Veber's rule[45]. Furthermore, pharmacokinetic properties were also evaluated based on various parameters such as blood-brain barrier permeation, human intestinal absorption capacity, and toxicity profiles.

Molecular docking

Molecular docking simulations were performed to investigate the binding interactions between chemical species found in the infusion extract of *A.halimus* and drug targets.

Polyphenols (gallic acid, p-coumaric acid, and protocatechuic acid) and flavonoids (catechin, isorhamnetin, and rutin) were selected as ligands. All the structures were optimized with DFT/B3LYP-6-31G(d,p) basis set using Gaussian 03 program. The crystal structures of the chosen drug targets were downloaded from the RCSB Protein Data Bank.

UCSF Chimera 1.14[68] software was used to prepare the target proteins and for molecular docking simulations. Before molecular docking, crystal water molecules and co-crystallized ligands were deleted from the target protein structures, and missing atoms were added. Polar

hydrogens were added and Gasteiger charges were assigned using the Dockprep tool[69,70]. Molecular docking simulations were performed using locally hosted AutoDock Vina software[71]. The active site in each protein target was defined based on the literature and then confirmed by selecting all co-crystallized ligands located at 6 Å from the center of the crystal pose in Biovia Discovery Studio 4.5.

Cyclooxygenases-1 COX-1 (PDB: 6Y3C) and cyclooxygenases-2 COX-2 (PDB: 5KIR) proteins were selected as targets for the *in silico* anti-inflammatory study. These enzymes play a key role in the inflammation process and they are inhibited by most NSAIDs[72].

All the ligands were flexibly docked into the active site of the biomacromolecules. The grid box parameters were defined as (-30.993, - 60.409, -1,077) and (29.889, 32.146, 55,298) for COX-1 and COX-2, respectively, and the grid map size was set equal to 65 Å for the three dimensions.

In the *in silico* anticonvulsant activity study, GABA transaminase was chosen as a target. The inhibition of this enzyme leads to a higher concentration of neuronal GABA which reduces the risk of convulsion and epileptic seizures. Porcine GABA-T structure (1OHV) was downloaded from the RSCB data bank and used as a receptor. The (XYZ) coordinates of the active site were defined as (12.071, - 4.896, 18.923) and the grid map size was set equal to 60 $Å^3$. All the substances were flexibly docked into the cavity of the target.

The resulting dock poses of the compounds docked into each protein were analyzed for their hydrogen bond, hydrophobic, and Van der Waals interactions with each receptor and were visualized using Biovia Discovery Studio Visualizer software.

Results

1. Acute toxicity study

Researching the substances thought to have medicinal potential requires conducting toxicological research. The study on acute oral toxicity was carried out according to the protocols specified in OECD guideline No. 420. The experimental mice were administered a single dose of 5000 mg/kg BW of the extract obtained from the aerial parts of *A. halimus* for testing purposes. Table 1 indicates that both the control and treatment groups showed comparable average body weight growth over the 14-day observation period. In addition, there were no fatalities, atypical clinical complaints, or significant visual abnormalities observed throughout the trial. According to the OECD guideline (No. 420), the LD50 of the plant extract under study was determined to be greater than 5,000 mg/kg BW.

Table 1 Body weight changes in mice following the oral administration of the aqueous extract

	Control (Mean±S.E.M)	**Extract (Mean±S.E.M)**	**P**
Body weight			
D0 (g)	21.46±0.06	22.08±0.21	NS
D7 (g)	23.84±0.48	24.58±0.49	NS
D14 (g)	26.02±0.23	26.46±0.42	NS

Changes in body weight			
D0−D7 (g)	2.38±0.43	2.5±0.29	NS
D0−D14 (g)	4.56±0.18	4.38±0.22	NS

D: Day, NS: no statistically significant

2. **Antipyretic effect**

The outcomes of the impact of the AEAH at doses of 250 and 500 mg/kg on the hyperthermia generated by brewer's yeast are presented in Table 2. The results demonstrate that both the PCM and the aqueous extract, administered at doses of 250 and 500 mg/kg, exhibited a significant reduction ($p < 0.05$ and $p < 0.01$) in hyperthermia starting from the second hour. The most significant reduction in hyperthermia occurred at the third hour for PCM ($p < 0.001$) and the aqueous extract at a dosage of 500 mg/kg ($p < 0.01$). The greatest reduction in hyperthermia occurs at the fourth hour when administering the aqueous extract at a dosage of 250 mg/kg. Nevertheless, there was no noteworthy decrease (ns = $p > 0.05$) in hyperthermia reported after 1 hour using PCM or AEAL (at doses of 250 and 500). mg/kg).

Table 2 Effect of AEAL on Brewer's yeast- induced pyrexia in mice

Treatment	Dose(mg/kg) body. weight	Rectal temperature in °c					
		−18h	0h	1h	2h	3h	4h
Control	-	36.18±1.04	38.25 ±1.02	38,03±0.45	38.38±0.24	38,35±0.47	38,45±0.42
PCM	150	36,23±1.12	38.73±1.01	37.11±0.35*	36.03±0.28***	36,03±0.42***	36,08±0.42***
AEAL	250	36,28±0.84	38.91±0.85	37,63±0.44*	37.03±0.34*	36,56±0.43***	36,43±0.36***
AEAL	500	36.26±0.56	38.61±0.78	37,36±0.24*	36,76±0.32***	36,06±0.32***	36,13±0.43 ***

Values are expressed as mean ± SEM (Standard error mean); n = 6 each group; *P <0.05, **P <0.01, ***P <0.001, significantly different compared to the control groups, data were analyzed by two-way ANOVA, followed by Tukey's (HSD) multicomparison test.

3. Effect of AEAL on INH-induced convulsions

As shown in Table 3, the mice were protected and the occurrence of seizures was effectively delayed by the administration of AEAL at doses of 250 and 500 mg/kg. In the model of seizures induced by Isoniazid. The AEAL treatment significantly prolonged the onset and duration of seizures induced by isoniazid in mice. The analysis of the results indicated that the oral administration of AEAL at a dosage of 250 mg per kilogram provided a 33.33% level of protection against convulsions generated by INH in mice. The administration of diazepam, a commonly used anticonvulsant medication, at a dosage of 10 mg per kilogram intraperitoneally, completely eliminated the convulsive symptoms caused by isoniazid in mice. Administering the AEAL at a dosage of 500 mg/kg had a substantial effect on delaying the start of convulsions ($p<0.01$) and reducing the length of convulsions ($p<0.05$) in mice that were induced to have convulsions by INH. Additionally, it effectively prevented death in mice experiencing convulsions caused by isoniazid.

Table 3 Effect of AEAL, and Diazepam on Isoniazid-induced convulsions in mice

Treatment	Dose (mg/kg)	Onset of convulsion (s)	Duration of convulsion (s)	No. convulsed / No. used	Animals not convulsed (% animals protected)	Mortality (%)
Control		46.16±.17	6.5±1.07	6/6	0	5/6
Standard (Diazepam)	10	0.00 ±0.00***	0.00 ±0.00***	0/6	100	0/6
AEAL	250	57.66±5.17***	3.11 ± 1.02*	4/6	33.34	1/6
AEAL	500	101.16±7.11***	2.68 ± 1.04**	1/6	83.33	0/6

Values are mean ± SEM (n=6); ns-p value not significantly different (compared with control using student's t-test), **P<0.01(compared with control using student's test), ***P<0.0001(compared with control using student's t-test).

4. *In vivo* hepatoprotective activity of AEAL against PCM-induced intoxication

The impact of AEAL pre-treatment on the serum biochemical markers, including ALT, AST, ALP, and TB, in rats intoxicated with PCM.

The administration of PCM resulted in a significant increase in AST levels by 146.8% ($p<0.001$), ALT levels by 53.09 % ($p<0.001$), ALP levels by 69.19 % and bilirubin by 237 ($p<0.001$) (Table 4). Mice that were treated with AEAL at doses of 250 mg/kg and 500 mg/kg, both before and

after treatment, showed a significant reduction in levels of AST, ALT, ALP, and bilirubin ($p<0.001$) compared to the controls that were given PCM. Compared to the lower dosages, the higher dose (500 mg/kg) exhibited superior hepatoprotective efficacy. The liver chemical biomarkers showed a decrease in the following order: ALP (85.71%), ALT (97.65%), AST (86.6%) and Total
Bilirubin (80.06%), at a dosage of 500 mg/kg. At a dosage of 200 mg/kg, ALP and AST experienced a reduction of 79.3% and 60.25, respectively, while ALT reduced by 96.09%.

Table 4 Effect of AEAL on serum liver enzymes (ALT, AST, and ALP) and, total bilirubin, in PCM-induced liver damage in mice.

Treatment	Dose (mg/kg)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dL)
Control		75.4 ± 0.22***	87.2 ± 0.64***	130.5 ± 0.25***	1.27 ± 0.20***
PCM	2000	203.6± 0.24	133.5 ± 0.24	220.8 ± 0.55	4.28 ±0.43
PCM+ silymarin	2000+200	78.6 ± 0.23*** 97.7	89.4 ± 0.29*** 95.2	145.9 ± 0.73*** 82.9	1.78 ± 0.24*** 83.05
PCM + AEAL	250	80.4 ± 0.11*** 96.09	105.6 ± 0.82*** 60.25	150.7 ± 0.67*** 77.63	2.00 ± 0.40*** 75.74
PCM + AEAL	500	78.4 ± 0.11*** 97.65	93.4± 0.52*** 86.6	143.4± 0.67*** 85.71	1.87 ± 0.48*** 80.06

Values are expressed in mean ± SEM (n=6) one-way ANOVA followed by Tukey-Kramer's test. Where, *represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$

5. Effects of AEAL on Antioxidant Biochemical Markers in the Liver

The administration of Paracetamol (PCM) resulted in a notable increase ($P < 0.001$) in liver oxidative stress, as evidenced by the large elevation of MDA levels. This was accompanied by a significant decrease ($P < 0.001$) in the activities of SOD, GSH, and CATA compared to the control group. The negative effects caused by PCM administration were significantly mitigated ($P < P\ 0.001$) when the mice were treated with either silymarin or AEAL, as compared to the PCM group. There was no notable distinction observed between the effects of silymarin and PCM on inducing oxidative stress in the liver. This stress was characterized by a significant increase ($P < 0.001$) in MDA levels, along with a significant decrease ($P < 0.001$) in SOD, GSH activities, and CATA levels, when compared to the control group (table 4). The harmful effects caused by PCM administration were greatly mitigated ($P < 0.001$) when the mice were treated

with either silymarin or AEAL, relative to the PCM group. There was no discernible disparity between the treatment of silymarin and AEAL.

Table 5 Effect of mung bean extracts on SOD, MDA, and GSH levels in liver homogenate of PCM induced acute liver toxicity in mice.

Treatment	Dose (mg/kg)	CAT mol of H2O2 decomposed/mg protein.	SOD b U/mg protein	MDA a nmol MDA/mg protein	GSH g/mg protein.
Control		110.42 ± 0.48***	27.23 ± 0.49***	1.65 ± 0.08***	28.45±0.04***
PCM	2000	67.08 ± 2.82	11.15 ± 0.47	4.98 ± 0.21	10.21±± 0.11
PCM + silymarin	2000+200	105.03 ± 2.14***	20.75 ± 1.20***	2.10 ± 0.10***	27.54±0.12***
PCM + extract	2000+250	94.43 ± 3.53***	19.37 ± 0.43***	1.83 ± 0.05**	24.46±0.02**
PCM + extract	2000+500	104.47 ± 2.37***	21.86 ± 0.53***	2.28± 0.18***	26.37±0.05**

Values are expressed in mean ± SEM (n=6) one-way ANOVA followed by Tukey-Kramer's test. Where, *represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, *** represents very significant at $p<0.001$

6. Histopathological results on hepatotoxic effects induced by PCM liver with or without pretreatment

The results of the histology (Figure 1) study supported the conclusions drawn from the biochemical analysis. The mice administered PCM demonstrated hepatic cell necrosis, which was characterized by nuclear loss and inflammatory cell infiltration. However, the mice that were administered either silymarin or *A.halimus* extract showed a significant improvement in thewere failed to protect the liver of mice from PCM-induced injury. The experiment found that the hepatoprotective effects of silymarin and *A.halimus* extract were similar.

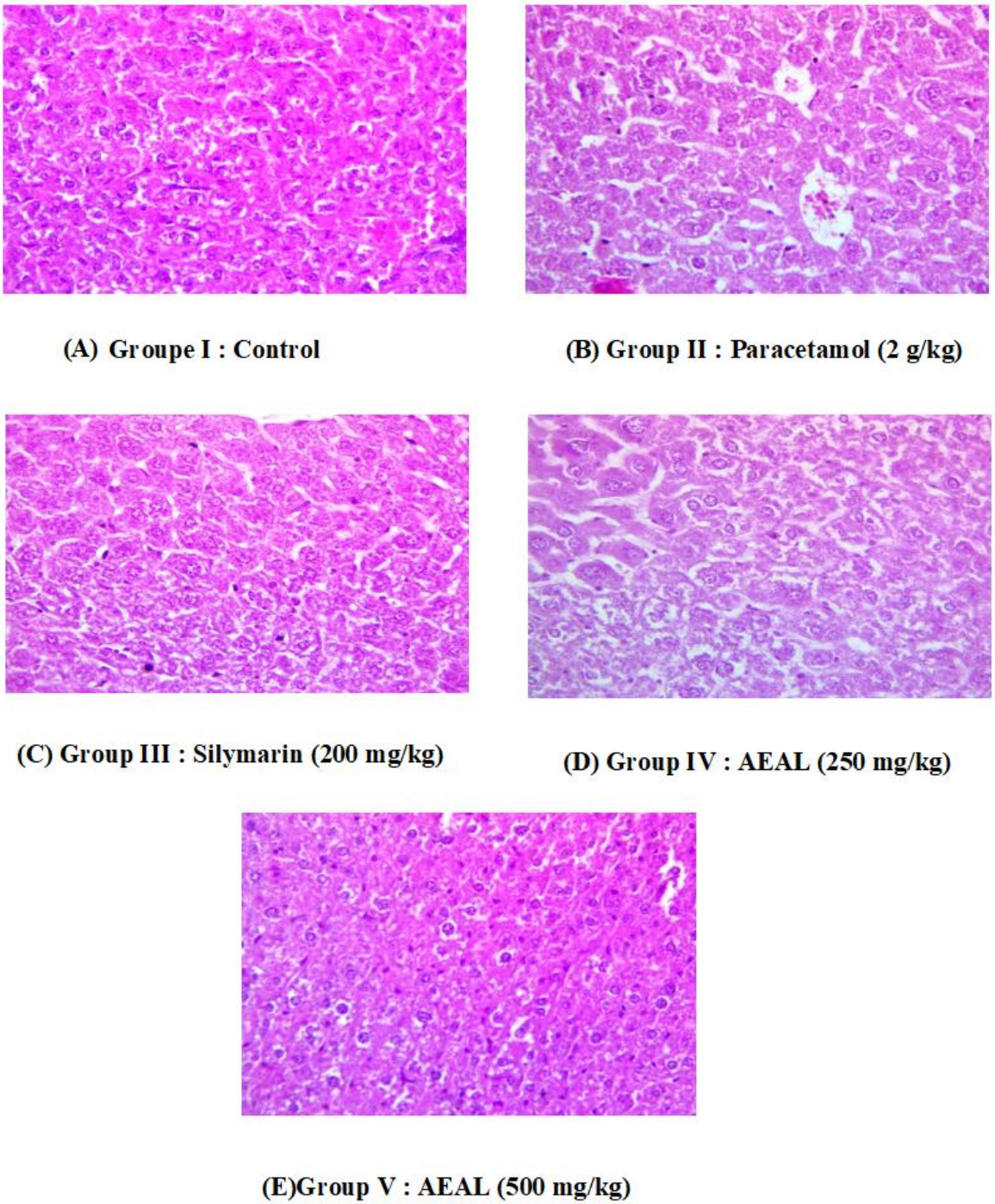

Figure 1. Histological examination of liver sections from different groups (A) Group I: normal arrangement of hepatocytes. (B) Group II: section of liver tissue of paracetamol-treated group showing massive coagulative necrosis, hemorrhage, and inflammation. (C) Group III: section of 250 mg/kg silymarin liver tissue pretreated in the liver followed by paracetamol, showing preservation of normal hepatocytes. (D) Group IV: section of liver tissue pretreated with 200

mg/kg AEAL followed by paracetamol, showing tissue necrosis and inflammation. (E) Group V: section of liver tissue pretreated with 500 mg/kg AEAL followed by paracetamol, showing normal histology with mild inflammation (40× magnification).

7. *In silico* pharmacokinetics and drug-likeness properties

The drug-likeness profile, pharmacokinetics, and toxicity of the main compounds found in the aqueous extract of *A. halimus* were predicted using SwissADME, admetSAR, and ProTox-II web servers. Polyphenols and flavonoids including protocatechuic acid, coumaric acid, gallic acid, catechin, isorhamnetin, and rutin were tested.

The results of the predictions as reported in Tables 6-7 show that all the studied chemical compounds, with the exception of rutin, are potentially absorbed by the skin and the body cells since their molecular weight is below 500 Da[45–47]. The tested polyphenols: protocatechuic acid, coumaric acid, and gallic acid, exhibit a good drug-likeness profile with good lipophilicity parameters as the predicted values of log P and TPSA are found in the acceptable range (-0.4 <logP< 5.6 and 60 <TPSA (Å²)< 150)[48,49]. In addition, they display an acceptable number of donors and acceptors of hydrogen bonds (HBA<5, HBD<10). These results indicate that the polyphenols have good oral drug bioavailability which is confirmed by Lipinski's and Veber's filters.

On the other hand, the studied flavonoids show poor oral bioavailability. Indeed, the values of TPSA and log P for isorhamnetin, and rutin were predicted to be out of the acceptable range while catechin showed acceptable lipophilicity. Additionally, all the flavonoids displayed an out-of-range number of hydrogen bond acceptors while only catechin showed an allowable number of hydrogen bond donors. Thus, isorhamnetin and rutin fail more than one of the conditions of Lipinski's rule and Veber's rule criteria. However, breaking these rules does not necessarily mean that potential drugs are ineffective as some commercial drugs have demonstrated effectiveness despite being out of the studied filters[50].

The predicted pharmacokinetic analysis shows that all the substances, except Isorhamnetin, display high gastrointestinal absorption with values lying between +0.556 and +0.995. It is worth noting that, the inhibition of PG protein leads to the interaction and accumulation of drugs within the cells and produces cell toxicity[51]. In our case, all the compounds were found to be non-inhibitors of PG.

In addition, the tested polyphenols and flavonoids are predicted to be nonpermeable to the Blood blood-brain barrier (BBB) which is preferable for non-central Nervous System (CNS) drugs[52].

Also, the results of the *in silico* study show the compounds potentially found in *Atriplex halimus* aqueous extract are non-CYP450 2D6 inhibitors and nor substrates which indicate a slow metabolism.

Besides, almost all the compounds were predicted as non-AMES toxic, non-carcinogens, and non-Human Ether-a-go-go-Related Gene (hERG) blockers.

Globally, the tested polyphenols and flavonoids exhibit an acceptable drug-like profile and good pharmacokinetic properties.

Table 6 Calculated Drug-Likeness properties of the main compounds found in AEAL

Property/rule	Protocatechuic acid	Coumaric acid	Gallic acid	Catechin	Isorhamnetin	Rutin
MW (g/mol)	154.12	164.14	170.12	290.27	478.40	610.52
Log P	0.66	1.26	0.21	0.83	0.05	-1.51
TPSA ($Å^2$)	77.76	60.53	97.99	110.38	199.51	269.43
HBA	4	3	5	6	12	16
HBD	3	2	4	5	7	10
Lipinski	Yes	Yes	Yes	Yes	No	No
Veber	Yes	Yes	Yes	Yes	No	No

Table 7 ADMET properties of the AEAL main compounds, predicted by admetSAR and Pro-Tox II web servers.

Property	Protocatechuic acid	Coumaric acid	Gallic acid	Catechin	Isorhamnetin	Rutin
%HIA	+ 0.959	+ 0.995	+ 0.892	+ 0.892	- 0.485	+ 0.556
PG Inhibitor/substrate	No/No	No/No	No/No	No/No	No/No	No/No
BBB	- 0.675	- 0.775	- 0.725	- 0.675	- 0.880	- 0.850
CYP2D6 substrate	No	No	No	Yes	No	No
CYP2D6 inhibitor	No	No	No	No	No	No
AMEST	No	No	No	Yes	No	Yes
Acute oral toxicity	Low (III)	Low (III)	Low (III)	Non-toxic (IV)	Low (III)	Low (III)
Carcinogenicity	No	No	No	No	No	No
hERG inhibition	No	No	No	No	No	No

8. Molecular docking results

Inhibition of cyclooxygenases COX-1 and COX-2

The anti-inflammatory and antipyretic properties of polyphenols and flavonoids found in the aqueous extract of *Atriplex halimus* were studied by evaluating the inhibition of cyclooxygenases

COX-1 and COX-2. These enzymes are the main targets of non-steroid anti-inflammatory drugs and their inhibition is often related to anti-inflammatory activities.

The obtained docking scores as reported in Tables S1-S2 indicate that all the tested substances bind spontaneously to the active site of the targets since the values of the binding energy are negative. In addition, the highly negative values (-10.1 to -8.5 kcal/mol) of the docking score obtained for the tested flavonoids (catechin, isorhamnetin, and rutin) suggest that these compounds could act as potent inhibitors of cyclooxygenases. In the case of the studied polyphenols (protocatechuic acid, coumaric acid, and gallic acid), the values of binding energy lie around -6.2 kcal/mol which suggests that they may be less efficient than the other tested bioactive molecules.

On the other hand, the docked poses of all the substances show that the tested ligands bind to the cavity of the COX-1 and COX-2 enzymes, mainly, through hydrogen bonding and hydrophobic interactions (Figures 2-3). Rutin displayed the highest number of favorable interactions including more than ten hydrogen bonds between the oxygen atoms of the flavonoid and protein residues such as Ala199 (d=2.11Å), Gln203 (d=2.43Å, 2.44Å, 2.73Å) for COX-1 and Gly225 (d=1.83Å) and Asn375 (d=2.40Å) for COX-2. In addition to hydrophobic physical bonds, pi-interactions are also observed between catechin and enzyme residues such as pi-pi stacking between the phenolic fraction of the bioactive molecule and the His386 residue of COX-1. Globally, the docked flavonoids exhibit various types of interactions with the receptors due to the presence of donor and acceptor sites which make them highly reactive.

The examination of the docked conformations of the polyphenols indicates that these compounds are stabilized in the targets' cavities via hydrogen bonds. Protocatechuic acid shows the highest number of favorable interactions among the polyphenols, it binds to the active site of COX-2 through four H-bonds involving its hydroxyl groups and His122, Leu366, Lys369, and Gln370 residues. Other interactions such as carbon-hydrogen bonds between the hydroxyl and carbonyl group of gallic acid and the His388 residue of COX-1 or the pi-cation interaction between the aromatic cycle of the polyphenol and Glu140 of COX-2.

It is worth mentioning that all the studied substances found in *Atriplex halimus* extract show no selectivity towards the receptors and could act as COX-1 and COX-2 inhibitors. Thus, the anti-inflammatory activity observed in the in vivo experiments is probably related to the inhibition of the studied cyclooxygenases.

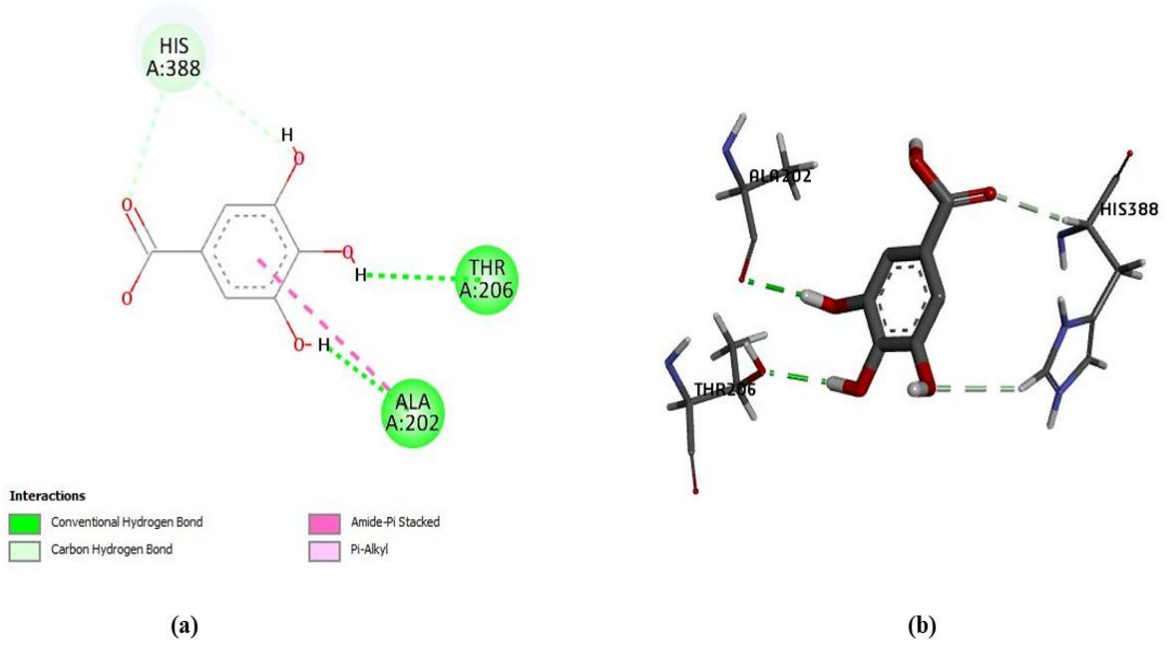

Figure 2. 2D (a) and 3D (b) conformations of gallic acid docked with COX-1

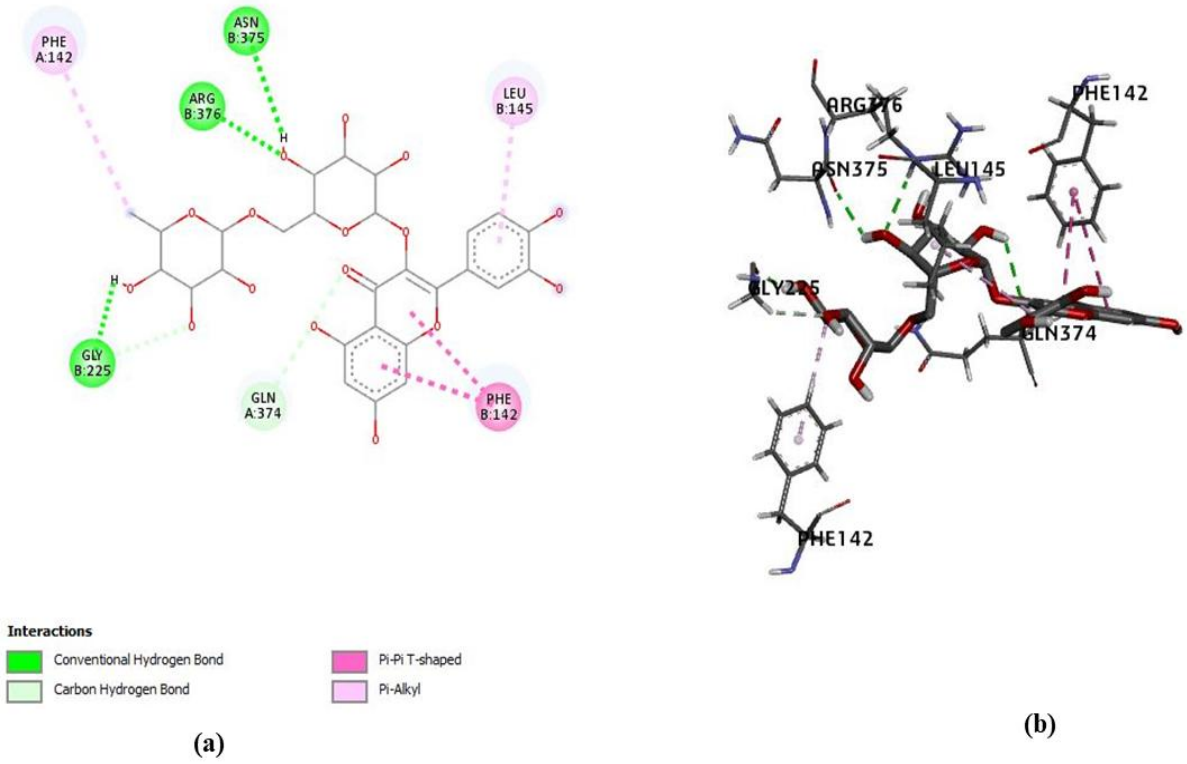

Figure 3. 2D (a) and 3D (b) conformations of rutin docked with COX-2

9. Inhibition of GABA transaminase (GABA-T)

GABA transaminase (GABA-T) is one of the main targets of epileptic drugs. This enzyme is involved in the breakdown of gamma-aminobutyric acid (GABA), which is a neurotransmitter in the brain. By inhibiting this enzyme, the breakdown of GABA is slowed down, leading to an increase in GABA levels in the brain. Since GABA is an inhibitory neurotransmitter, higher levels can help reduce the excessive neuronal activity that occurs during seizures, thus serving as a therapeutic approach in epilepsy treatment.

In our study, we aimed to study the inhibitory potential of GABA-T by the main compounds found in the *Atriplex halimus* extract using molecular docking. The obtained results are reported in Table S3 (see supplementary data).

The values of the docking score of all the tested bioactive molecules with GABA-T enzyme are found to be highly negative which indicates that these compounds are spontaneously linked to the active site of the receptor. Additionally, the studied flavonoids show the most favorable energy binding values suggesting that these substances are more likely to act as efficient inhibitors of GABA-T.

According to their docked conformations (Figures 4-5), all the tested compounds were found to be stabilized in the target's cavity through favorable hydrogen bonds. Polyphenols such as gallic acid and p-coumaric acid H-bind to various residues such as Gln38, Asn39, and Ile350 in addition to being involved in pi-alkyl interactions.

On the other hand, in addition to the stabilizing hydrogen bonding, the tested flavonoids were found to be stabilized in the GABA-T's cavity by various pi-interactions. The aryl group of catechin is involved in pi-pi stacking interaction with the Thr348 residue while the 4H-chromen-4-one fragment of isorhamnetin is docked to the active site with additional pi-charge interactions with Lys203 and Glu270 residues. In the case of rutin, its flavone moiety is involved in pi-pi stacking and pi-charge interactions with various residues from the cavity of the receptor including Lys203, Glu270, Tyr348, and Phe351.

It is worth mentioning that all the substances are docked in the same site as the co-crystallized ligand, Vigabatrin, a drug used as a GABA-T inhibitor to treat epilepsy. These results indicate that the compounds found in the aqueous extract of *A.halimus* have a great potential to inhibit GABA transaminase which probably led to the antiseizure activity observed *in vivo* in mice.

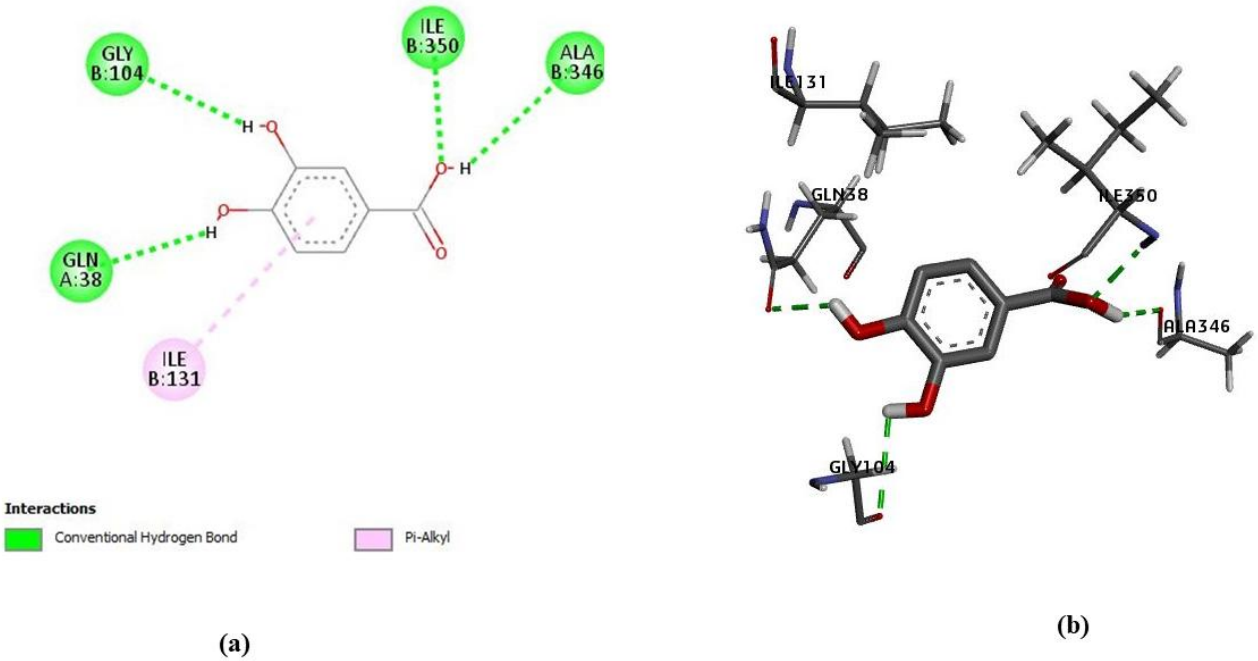

Figure 4. 2D (a) and 3D (b) conformations of protocatechuic acid docked with GABA-T

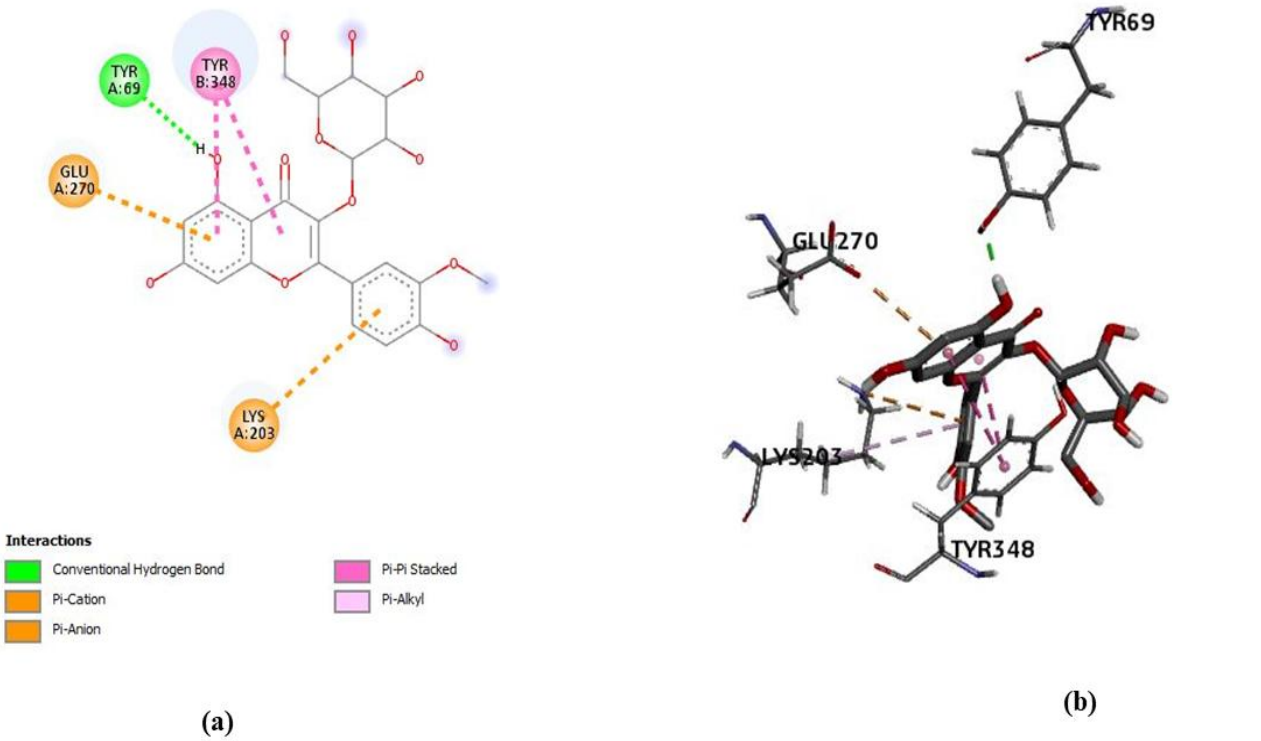

Figure 4. 2D (a) and 3D (b) conformations of isorhamnetin docked with GABA-T

Discussion

Herbs are increasingly recognized as a valuable source of supplementary medicine in healthcare practices worldwide. Consumers generally do not give high importance to the efficiency and safety of the dietary supplements they routinely consume. When evaluating the toxicity of the plant extract from *Atriplex halimus*, mortality is an important factor as it indicates the immediate outcome of severe toxicity[29]. No fatalities or pathological abnormalities were detected throughout the acute toxicity evaluation. The AEAL oral LD50, which exceeds 5,000 mg/kg BW, is categorized as either category five or unclassified in the Globally Harmonized System[30]. The LD50 test indicates that the AEAL is not hazardous. Yeast-induced or examining the fever caused by different pathogens offers a cost-effective and appropriate approach to studying potential antipyretic medications[31]. This method establishes a connection between the occurrence of proteins in yeast infections and the occurrence of fever through inflammatory responses[32]. This method establishes a correlation between the presence of proteins in yeast infections and the occurrence of fever through inflammatory responses. Moreover, the secretion of cytokines such as interleukin-1β (IL-1β) and IL-6, interferon-α (IFN-α), and tumor necrosis factor-α (TNF-α), together with prostaglandins like PGE2 and PGI2, activate the brain and lead to an increase in body temperature[33,34]. Studies have found that several antipyretic medications, including PCM which was employed in this study, work by suppressing the activity of the cyclooxygenase enzyme. This leads to a decrease in the levels of PGE2 in the hypothalamus area. However, alternative mechanisms of fever control cannot be ruled out[34]. The AEAL comprised phytochemical constituents. The antipyretic actions of steroids and flavonoids have been proven in numerous studies[35]. It has also been found by numerous investigations that medicinal herbs with antinociceptive properties also have antipyretic and anti-inflammatory properties[31]. The reason for this may be the mechanism that suppresses pain, fever, and inflammation, as there is a correlation between the suppression of inflammatory mediators in each of these cases. This further demonstrates the assessment of this plant's potential for anti-inflammatory properties.

Isoniazid (INH) is an anti-tubercular drug that can induce seizures when used in higher doses. This phenomenon arises due to the interference of INH with the synthesis of GABA, a neurotransmitter that plays a crucial role in the regulation of brain activity. INH specifically inhibits the cofactor pyridoxal-5-phosphate, which is necessary for the enzyme Glutamic acid Decarboxylase (GAD) to function. GAD is the enzyme that catalyzes the conversion of glutamic acid into GABA. Animals treated with AEAL had a significant effect on seizures caused by INH, possibly due to an improvement in the GABAergic pathway in the central nervous system (CNS). Diazepam is a commonly prescribed anticonvulsant drug that specifically acts on the beta subunit of the GABA-A receptor. It functions by augmenting the activation of the GABA-gated chloride channel, hence inhibiting excessive neuronal activity and seizures. AEAL treatment demonstrated a notable impact on convulsions generated by INH in animals, which may be associated with an enhancement in the GABAnergic pathway in the central nervous system. Flavonoids could contribute to the anti-epileptic effects of AEAL.To assess the effectiveness of a potential hepatoprotective medication, several researchers have used protection against PCM-induced toxicity as a test[36].

Paracetamol (PCM) is a commonly prescribed medicine that alleviates pain and lowers fever. Multiple studies have demonstrated that high doses of PCM can induce liver cell necrosis in animals[37]. To evaluate liver damage, the concentrations of biochemical indicators such as ALT, AST, ALP activity, and serum bilirubin are analyzed. In our investigation, we confirmed the hepatotoxicity induced by PCM by seeing elevated levels of biochemical markers including ALT, AST, ALP, and total blood bilirubin, along with a significant reduction in total protein. This phenomenon can be attributed to the existence of several enzymes and a wide range of metabolic processes in liver cells. Elevated amounts of ALT were observed throughout the cytoplasm, whereas AST was mostly confined within the mitochondria. A rise in the ALT is usually accompanied by a simultaneous rise in the levels of AST, which play a vital role in the conversion of amino acids to keto acids[38]. Hepatotoxicity impairs the transport function of hepatocytes, leading to the leakage of the plasma membrane[39]. Consequently, these enzymes escape, resulting in an increase in their concentration in the bloodstream. Increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in liver damage caused by paracetamol (PCM) are indicative of hepatocellular leakage and impaired membrane integrity. Both silymarin and extract treatment significantly mitigated the increased levels of ALT and AST induced by PCM. These results were obtained by stabilizing the plasma membrane and healing the damage to liver cells[40].

The increased presence of alkaline phosphatase in the blood is caused by the cells lining the bile canaliculi producing more of it, in response to higher biliary pressure and cholestasis[25,26]. The etiology of hyperbilirubinemia was attributed to an overabundance of heme degradation and blockage of the intrahepatic bile duct. As a result, there is a notable inhibition of the process of amalgamating chemicals and the elimination of unconjugated bilirubin from damaged liver cells. Pre-administration of either silymarin or extract effectively controlled alkaline phosphatase activity and bilirubin level, suggesting an enhancement in the secretion mechanism of liver cells[41].

A reduction in catalase activity might result in numerous detrimental effects due to the accumulation of highly toxic metabolites and hydrogen peroxide following the administration of PCM. The accumulation of substances may lead to oxidative stress within the cells[20]. Administering extracts of *A. halimus* at doses of 250 and 500 mg/kg improves catalase activity in mice, hence limiting the excessive accumulation of free radicals and protecting the liver against PCM poisoning. Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, are essential for protecting organisms against reactive oxygen species. Superoxide dismutase (SOD) is an enzyme that participates in defense mechanisms by facilitating the conversion of superoxide radicals into hydrogen peroxide. Catalase is a protein that contains heme and is found in the peroxisomes of eukaryotic cells. The primary purpose of this enzyme is to expedite the transformation of hydrogen peroxide into water and oxygen. The decrease in glutathione, superoxide dismutase (SOD), and catalase enzyme activity indicates the existence of toxic effects resulting from reactive oxygen species produced by noxious chemicals and proteins. The hypothesis of lipid peroxidation has been suggested[42].

Glutathione (GSH) neutralizes reactive oxygen species, such as hydrogen peroxide and Superoxide radicals are neutralized by this substance, which also preserves the integrity of membrane protein thiols. The primary factor responsible for liver damage caused by PCM is the depletion of glutathione (GSH) levels in liver mitochondria. The group that received PCM exhibited reduced levels of GSH. The presence of this phenomenon can be ascribed to the interaction between GSH and NAPQI, resulting in the formation of mercapturic acid[13]. EXTRACT markedly reduced the levels of MDA compared to the PCM-treated group, hence ameliorating and reversing the hepatotoxic effects of PCM on liver tissue. The phytochemical constituent families of flavonoids, saponins, and tannins are widely acknowledged for their hepatoprotective and antioxidant properties[43,44].

The outcomes of the histology study supported the biochemical analysis' conclusions. The mice exposed to PCM had hepatic cell necrosis, characterized by nuclear depletion and infiltration of inflammatory cells. However, the mice that were administered either silymarin or *A.halimus* extract showed a significant improvement in the viability of their liver cells. Both the inquiry and analysis demonstrated that both silymarin and *A.halimus* extract displayed similar hepatoprotective characteristics.

5. Conclusions

In conclusion, the study showcased significant antipyretic and anticonvulsant characteristics. Based on a study examining hepatoprotective characteristics, it has been found that the aqueous extract of *Atriplex halimus* leaves can restore liver function and reduce oxidative stress markers to normal levels, thereby safeguarding the liver. The investigation of the therapeutic characteristics of *A. halimus* extract can serve as a medicinal substitute for managing fever and averting convulsive episodes and hepatic complications associated with the use of synthetic medications.

Declarations

Ethical Approval

All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14).

Competing interests

The authors have no conflicts of interest to declare**.**

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Availability of data and materials

Not applicable.

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