Acute and Chronic Toxicity, Antioxidant *(in Vitro* and *in Vivo*), and Cytotoxic Effect of *Peganum Harmala* L. Hydromethanolic Seeds Extract Safety Profile and Biological Activities of *Peganum harmala* L

Dehiri Mounira (mounira.dehiri@univ-bba.dz)
Laboratory of Characterization and Valorization of Natural Products, Faculty of Nature and Life Sciences, University El-Bachir El-Ibrahimi, Bordj Bou Arreridj, 34000, Algeria.

Diafat Abdelouahab
Laboratory of Characterization and Valorization of Natural Products, Faculty of Nature and Life Sciences, University El-Bachir El-Ibrahimi, Bordj Bou Arreridj, 34000, Algeria.

Fatmi Widad
Laboratory of Health and Environment , Faculty of Nature and Life Sciences, University El-Bachir El-Ibrahimi, Bordj Bou Arreridj, 34000, Algeria.

Deghima Amirouche
University of Bejaia

Riadh Ben Mansour
National School of Engineering of Sfax (ENIS)

Khalil Rebai
Laboratory of Characterization and Valorization of Natural Products, Faculty of Nature and Life Sciences, University El-Bachir El-Ibrahimi, Bordj Bou Arreridj, 34000, Algeria.

Mohamed Bouaziz
National School of Engineering of Sfax (ENIS)

Benbacha Faycel
Hospitalo- Public Establishment (HPE) of Bordj Bou-Arraridj, 34000, Algeria.

Research Article

Keywords: Peganum harmala L, antioxidant activity, cytotoxic effect, acute toxicity, chronic toxicity

Posted Date: October 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-982660/v1

Abstract

Peganum harmala L. (Harmel) is a widely used plant in the traditional Algerian medicine. The objective of the present study was to assess the safety profile of the *P. harmala* hydromethanolic seed extract (PhHm). The acute toxicity of PhHm extract was carried out based on OECD guidelines 425, where the extract was administered to mice by gavage in single doses. While in the chronic study, the extract was administered orally for 90 days in rats. The antioxidant (*in vitro* and *in vivo*) and antiproliferative activities of PhHm extract were also investigated. In the acute and chronic toxicity, the extract did not cause any behavioral changes and any mortality. In both study a significant increase in liver enzymes was observed while urea and creatinine levels decreased in the treated animals. In the repeated oral administration of PhHm extract, an increase of the platelet numbers was noted and the histological examination of the liver showed a leukocyte infiltration and a vascular congestion. For kidneys, the presence of glomerular and modular congestion was also observed and sinusoidal dilation in chronic toxicity. The PhHm extract was more cytotoxic to HeLa cell line and exhibited potent antioxidant *in vitro* and *in vivo*. It can be suggested, based on the results of this study that the PhHm seed extract was moderately toxic in acute administration (LD_{50} of 501.47 mg/kg) and the use of this extract is safe at doses ≤ 100 mg/kg. *P. harmala* could be a potential source of bioactive compounds with antioxidant and antiproliferative potential.

1. Introduction

Over the past few decades, the popularity of plant remedies has become more substantial and vital in various parts of the world. These medicinal plants can therefore constitute important resources for new substances with therapeutic potential. Plants are a rich source of medicinally active components, including phenols, flavonoids, volatile oil, etc. displaying several pharmacological effects and health benefits (Yu et al., 2021). They have been reported to provide protective actions by acting as anticancer, anti-inflammatory and anti-apoptotic agents (Qiu et al., 2020). Polyphenols of medicinal plants are powerful antioxidants capable of fighting free radicals (Reactive Oxygen Species - ROS), that are involved in the appearance of several pathologies such as cancer, diabetes, Alzheimer's disease, and cardiovascular disorders (Yeung et al., 2020).

The efficacy of a substance in pharmacology is not sufficient to justify its possible introduction into therapy. It is therefore necessary to define the benefit to risk ratio in the therapeutic indication of each substance (Antonious et al., 2006). Despite the medicinal benefits of plants, some of the phytochemical compounds might pose lethal threats to humans. Toxicity assessment is an approach to assess the adverse effects of items intended for use or consumption by humans. It must guarantee the safety of medicinal plants; ensure phytotherapy and the consumption of plants.

Peganum harmala L, commonly known as “Harmal” is a wild-growing flowering plant that belongs to the zygophylaceae family. This plant grows spontaneously in semiarid rangeland, steppe areas and sandy soils (Sepideh et al., 2021). *P. harmala* L. is one of the most important plants used in Algerian phytotherapy to cure many disorders. The seeds of *P. harmala* L. have pharmacological activities including; antibacterial effects, vasorelaxant, antihemosporidian, anticancer (Jalali et al., 2021), antinociceptive, antitumor and antiprotozoal effects (Mohamed Ahmed., 2020; Çelik et al., 2021). However, to our knowledge, no report is available on both the safety profile and antioxidant activities and cytotoxic effect of hydromethanolic extract from the seeds of *P. harmala* L. Based on that, the aim of the present study was to evaluate the acute and chronic oral toxicity and to investigate the pharmacological potentials of *P. harmala* hydromethanolic seed extract including its antioxidant (*in vitro* and *in vivo*) and antiproliferative activities.

2. Materials And Methods

2.1. Plant material

Peganum harmala L. (Zygophylaceae) seeds were collected from El-Hammadia, Bordj Bou Arreridj, Northeast Algeria, during the flowering stage (August 2018). A voucher specimen (N° AB-65, 2018) was identified by Pr Boudjelal Amel a botanist from Mohamed Boudiaf University - M'Sila, Algeria. The seeds of *P. harmala* L. were dried in dark, at room temperature for two weeks and ground into a fine powder using an electric mill.

2.2. Experimental animals

The *in vivo* study was performed on *Swiss albino* mice, females (20 to 30 g) for the study of acute toxicity and *Albino Wistar* female rats (150 and 200 g) for the chronic toxicity. All experimental animals were purchased from the Pasteur Institute (Algeria) and housed in plastic cages under normal laboratory conditions (12/12h light/dark cycle, 23 ± 2°C*)* and had free access to standard commercial diet (croquettes, Ets ONAB El-Kseur, Bejaia) and tap water. These animals were allowed an adaptation period of one week before any experiment. The experiments were conducted in accordance with the internationally accepted guidelines for evaluating the safety and efficacy of plant medicines (OECD, 2008).

2.3. Phenolic compounds extraction

To obtain the *P. harmala* L. hydromethanolic extract (PhHm), the dried powdered seeds were extracted three times by macerating in a methanol-water mixture (85%: v/v) at room temperature during 72 h, to allow maximum extraction of bioactive molecules (Markham, 1982). The resulting extract was filtered through a Whatman N°3 filter paper and was concentrated under vacuum in a rotary evaporator. The obtained residues were kept at 4°C for further analysis.

2.4. Colorimetric estimation of phenolic compounds

The total phenolic content (TPC) of PhHm extract was measured spectrometrically using the Folin-Ciocalteu method (Li et al., 2007). Gallic acid (20–140 µg/mL) was used for the standard calibration curve and the results were expressed as µg Gallic acid equivalents per mg of extract (µg GAE / mg of extract). The amount of flavonoids was determined by trichlorure d'aluminium ($AlCl_3$) method (Bahorun et al., 1996), based on the formation of a complex between flavonoids and aluminum chloride. Quercetin (1–40 µg/mL) was used as standard for calibration curve. According to the method of Kosalec et al. (2004), flavonols determined from the standard quercetin curve. Flavonoids and flavonols contents were expressed as µg quercetin equivalent per mg of extract (µg QE / mg of extract).

2.5. *In vitro* Antioxidant activity

2.5.1. DPPH and ABTS radical scavenging assay

The DPPH (Burits and Bucar, 2000) and ABTS free radical scavenging activity (Re et al., 1999) of PhHm extract were used for antioxidant activity estimation. BHT, BHA, quercetin and trolox were used as standards. The scavenging activity was calculated using the following equation:

$$I\ (\%) = [(A_c - A_s)/A_c] \times 100 \quad (1)$$

Where, I is the inhibition percentage, Ac and As are the absorbance of the control and the sample, respectively. The results were expressed as IC_{50} (concentration providing 50 % inhibition) and in µmol g^{-1} of Trolox-equivalent antioxidant capacity (TEAC) for DPPH and ABTS radical scavenging activity, respectively.

2.5.2. β-carotene bleaching assay

The antioxidant capacity of PhHm extract was also evaluated by measuring the inhibition of the volatile organic compounds and the conjugated diene hydro-peroxides arising from linoleic acid oxidation according to the method of Dapkevicius et al. (1998). Synthetic antioxidant BHT was used as positive control and methanol and distilled water were used as a negative control. The inhibition percentage of β-carotene oxidation was measured as follows:

$$AA\% = (Abs_{test} / Abs_{BHT}) \times 100 \quad (1)$$

The values of AA % are calculated after 24h and the results were expressed as IC_{50}.

2.5.3. Iron chealtion assay

To evaluate extract-iron interaction, the ferrozine test was performed following the method of Decker and Welch, (1990) modified by Le et al. (2007). The red chromophore of the Fe^{+2}-ferrozine complex was measured spectrophotometrically at 562 nm against

a blank. EDTA was used as reference chelator and the inhibition percentages were determined according to the previous formula (1).

1.1. Antiproliferative activity

1.1.1. Cell lines and culture condition

HeLa cells (cervical cancer line, adherent) were used to investigate the cytotoxicity effect of PhHm extract. This cell line was grown in RPMI 1640 medium (Gibco) supplemented with 10% (v/v) foetal calf serum (FCS) and 2 mM L-Glutamin in tissue culture flasks. They were kept at 37°C in an incubator at 5% CO_2 and saturated with water vapor 95%.

1.1.2. MTT test

The antiproliferative and cytotoxic activity of the extract against HeLa cell lines was evaluated *in vitro* by the method of Mosmann, (1983) using MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). HeLa cells (4.44 x 10^5 cells/well) were incubated in 96-well cell plates for 24 hours in the presence or absence of extract (10 µL). 10 µL of MTT solution (1 mg/ml in PBS) were added to each well and incubated for 4 h at 37°C in a CO_2-incubator. A volume 180 µL of the medium was removed from every well without disturbing the cell clusters. Formazan crystals were dissolved using 180 µL methanol/DMSO solution (50:50), and the preparations were thoroughly mixed on a plate shaker for 30 min in the room temperature. Finally, after the dissolution of all crystals, the absorbance of each well was determined with a microplate reader (ELx 800) at 570 nm. The results were expressed as a percentage inhibition of cell proliferation and calculated according to the following formula:

% Inhibition= 100 - % viability (2)

Where: % viability = $(DO_{extract}*100)/Do_{control}$

The 50% inhibition concentration (IC_{50}) of the extracts was determined as the drug concentrations that reduced cell number by 50% in treated compared to untreated cultures.

1.2. Acute toxicity

The acute toxicity was evaluated according to guidelines 425 of the Organization for Economic Cooperation and Development (OECD, 2008) with slight modifications. The animals were divided into seven groups (8 mice/group). The PhHm extract was freshly dissolved in distilled water (4% DMSO) and administered as a single dose by gavage at 2000 mg/kg body weight (B.W). The mortality was observed for 15 days. The dose administered is considered as toxic, if two or three deaths are registered (OECD, 2008). If no mortality is observed at this dose, the same procedure will be repeated for the dose of 5000 mg/Kg. In parallel, eight animals were treated with the vehicle (control group). The results of this test allow an approximation of the LD_{50}. For the determination of the LD_{50} value, five other doses were used (800-500-300-200-70 mg/kg) (Rasekh et al., 2010). The LD_{50} value was estimated by computerized techniques (Abu Sitta et al., 2009).

1.2.1. Clinical observations

The general behavior of mice was continuously monitored for the first hour after the administration, and periodically during the first 24 h and every day for 14 days. All parameters were recorded such as food and body weights at the beginning, then once after every seven days during the study. Observation was carried out focusing attention on animal response and general physical condition (somnolence, twitch, paralysis, convulsion and unusual locomotion). Hair characteristics and possible alterations in feces and urine coloration were also observed.

1.2.2. Organ’s weight, plasma preparation and biochemical analysis

After the sacrifice of animals, the organs (liver, spleen, kidneys and heart) were quickly removed and weighed individually. The relative organ weight of each animal was then calculated relating the absolute organ weight (g) and body weight (g) of the animal on the day of sacrifice. Blood samples were collected in heparin-tubes and then centrifuged at 3000 rpm for 10 min.

Plasma biochemical parameters: urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were analyzed using a spectrophotometer and commercial kits (Spinreact, Spain).

1.2.3. Histological analysis

Liver and kidneys were fixed in formalin (10 %) and embedded in paraffin blocks. Sections of 5 µm thick were made by using rotary microtome and stained with hematoxylin and eosin. The slides were examined microscopically for observing the pathological changes (Martey et al., 2010). The analysis of the different organs (liver, kidneys) was performed using an Optika B-500T I-5 microscope (10 and 40×).

1.3. Chronic toxicity and *in vivo* antioxidant activity

1.3.1. Animals' treatment

The study was conducted according to OECD Guideline 407 (OECD, 2008b). The PhHm extract was administered by gavage daily for 90 days. The animals were randomly distributed into four groups of eight animals each: Group C: served as a control and received 4% DMSO solution, Group Vit C: received 100 mg/kg of Vit C, Group D1, Group D2 and Group D3: received the PhHm extract in 100, 200 and 300 mg/kg B.W, respectively. During the administration period, rats were closely monitored for any evident clinical signs of toxicity including acute pain, respiratory disorder, and appearance of stools, mobility disorders and death. The weight was noted before treatment (day 0) and every week throughout the study period.

1.3.2. Collection of blood and plasma

At the end of each month, the rats were fasted overnight, but allowed access to water. Blood samples were obtained by retro-orbital puncture (Waynforth, 1980) and collected in tube containing EDTA (for hematological parameter analysis), and tube containing heparin. For biochemical analysis and plasma antioxidant activity using DPPH scavenging assay. The hematologic analysis was carried out by a Coulter automate (Akmoum laboratory, Bordj Bou Arreridj), and biochemical tests were carried out using commercial kits (Spinreact, Spain).

1.3.3. Organs weight, histological analysis and preparation of tissue homogenates

After sacrifice, livers and kidneys were removed, washed by NaCl (0.9 %) and weighted. Each animal liver was divided into two pieces: one piece was fixed with kidneys in 10% formalin for histological analysis and the last one was immediately stored at -20°C until further antioxidant analyses. To prepare 10 % liver homogenate using ice cold KCl (0.15 %, w/v). Then, the homogenate was centrifuged twice at 4000 rpm/min for 10 min at 4°C to purge cellular debris and supernatant was collected and stored at -20°C until quantification of antioxidant enzyme; catalase activity (CAT), reduced glutathione (GSH) and malondialdehyde (MDA). Total protein content in the supernatant was measured using commercial kits (Spinreact; Madrid, Spain).

1.1.1. In vivo antioxidant activity

Plasma ability to scavenge DPPH radical was measured using the method of Cuendet et al. (1997) by mixing 25 µL of plasma with 625 µL of DPPH (0.004%). The plasma antioxidant capacity (CAP) was then calculated. The activity of CAT was calculated by taking the extinction coefficient of H_2O_2 to be 43.6 $M^{-1}cm^{-1}$ at 240 nm and expressed as µmole of H_2O_2 consumed/min/mg protein (UI/mg protein) (Claiborne, 1986). Reduced glutathione (GSH) levels were determined in the homogenates of liver using Ellman's reagent (5, 5 0- dithiobis-(2-nitrobenzoic acid), DTNB) (Ellman, 1959). The amount of GSH present in the tissue was calculated using TNB extinction coefficient to be 1.36 x 10^4 $M^{-1}cm^{-1}$ at 412 nm and expressed as µ moles /g tissue. Thiobarbituric acid reactive substances (TBARS) assay was used to assess MDA level in liver (Okhawa et al., 1979). The concentration of MDA was calculated using MDA-TBA adduct extinction coefficient to be 1.56 x 10^5 $M^{-1}cm^{-1}$ at 530 nm and expressed as nmoles MDA/g tissue.

1.2. Statistical analysis

The results are expressed as mean ± SD of triplicate measurements (*in vitro* study) and as mean ± SEM of triplicate measurements (*in vivo* study). GraphPad Prism Software (version 8.00) was used for statistical analysis. Data were analyzed using Student's t-test and one-way analysis of variance (ANOVA), followed by Dunnett test for multiple comparison. The differences were considered significant at $p<0.05$.

3. Results

3.1. Quantitative analysis of phytochemicals

Polyphenols, flavonoids and flavonols are very important compounds in plants because their therapeutic interest and their antioxidant activity has attracted most attention. The results obtained in this study showed a high level of polyphenols (114.1 ± 0.90 µg of GAE / mg of extract), flavonoids (31.20 ± 1.45 µg of QE / mg of extract) and flavonols (95.40 ± 0.80 µg of QE/mg of extract) compounds in the PhHm extract.

3.2. *In vitro* antioxidant activity

From the IC50 values obtained, the extract exhibits a high DPPH scavenging activity (52 ± 2.033 mg/mL) which is lower than BHT (28 ± 0.17 mg/mL), BHA (4 ± 0.001 mg/mL) and quercetin (1.1 ± 0.011 mg/mL) used as standards antioxidants ($p < 0.01$). Similarly, to DPPH assay, ABTS assay revealed that PhHm extract exerted a significantly higher antioxidant capacity. The PhHm extract presented good potential to neutralize ABTS radical (1.48 ± 0.01 µmol g^{-1} TEAC).

Analysis of antioxidant capacity evaluated with β-carotene bleaching assay revealed a high capacity of PhHm extract to prevent the bleaching of β-carotene after 24 h of incubation (76.18 ± 0.21%) as compared to the positive control (89.11 ± 0.74%). PhHm extract exhibited potent chelating activities (IC50: 1.184 ± 0.03 mg/mL), but remained inferior to that of EDTA (IC50: 0.023 ± 0.05 mg/mL) ($p < 0.01$).

3.3. Antiproliferative activity

The effect of different concentrations of the extract on HeLa cells survival was studied using the MTT reagent. PhHm extract were very active, as it has reduced HeLa cells viability to 20.48% at 0.015 mg/mL, while at the dose 2 mg/mL almost all HeLa cells were destructed (95%). Cytotoxicity was expressed as the concentration of extracts inhibiting cell growth by 50% (IC_{50}). The IC_{50} values of PhHm extract was evaluated to 0.031 ± 0.007 mg/mL.

3.4. Acute toxicity

Toxicity analysis is a fundamental step to be undertaken for any preparation intended to be used in humans. This study revealed that mice death after oral administration of PhHm extract was dose dependent. The administration of the extract with doses ≤ 300 mg/kg did not cause any behavioral changes and any mortality during the study period, mice deaths gradually reached 100% at 2000 mg/kg. The median lethal dose (LD_{50}) of PhHm extract was estimated to be 501.47 mg/kg B.W. Some adverse effects, such as hypo-activity, anorexia and diarrhea were observed just after the oral administration. The groups treated with 70, 200 and 300 mg/kg doses had normal body weight (no significant decrease, $p >0.05$) during the first and second weeks compared to the control group. However, groups receiving the 500 and 800 mg/kg doses showed a significant ($p<0.01$) reduction in body weight during the same period (Fig. 1). The oral administration of PhHm extract did not cause any significant changes in the relative weight of the organs (liver, spleen, kidneys and heart) in the treated groups compared to the control group (Table 1).

Table 1
Organs relative weight of mice treated with PhHm extract and control group.

Groups	Control group	800 mg/kg	500 mg/kg	300 mg/kg	200 mg/kg	70 mg/kg
Liver	50.77 ± 4.47	59.94 ± 6.6[a]	50.3 ± 5.6[a]	49.4 ± 3.3[a]	52.01±2.9[a]	50.3± 2.4[a]
Heart	6.11 ± 0.54	5.76 ± 0.53[a]	5.06 ±0.07 [a]	4.8± 0.09 [a]	6.3± 0.40 [a]	5.4± 0.38 [a]
Kidneys	7.02 ± 0.79	7.43 ± 2.26 [a]	6.19 ± 0.2 [a]	5.6±0.52 [a]	5.4± 0.28 [a]	5.13 ± 0.2 [a]
Spleen	5.52 ± 0.86	3.92 ± 6.48 [a]	4.4 ± 0.18 [a]	3.2± 0.30 [a]	4.9± 0.37 [a]	3.9 ± 0.28 [a]
Values expressed as mean ± SEM, n=8 animals /group. a: not significant.						

In the current study, the enzyme AST did not present any significant variation when compared to the controls. The ALT and ALP in the group of 800 mg/kg showed a significant increase (*p<0.01*) compared to the control group (Fig. 2). In the present study, a significant (*p<0.01*) dose-dependent decrease in creatinine was observed. The histological examination of the liver revealed a vascular congestion and the leukocyte infiltration in some tissues (500 and 800 mg/kg).

The results of the histological examination of kidneys at 70 mg/kg revealed no architectural alteration (lobular and tubular) compared with control group except the presence of glomerular and medullar congestion in some tissues of 800 mg/kg treated mice (Fig. 3).

3.5. Chronic toxicity and *in vivo* antioxidant activity

The administration of extract (100, 200, and 300 mg/kg) to rats did not cause any behavioral changes, visible symptoms of toxicity or mortality in animals during the entire 90-days the observation period. During the first month, a non-significant weight loss was noted in all treated groups. A significant decrease in body weight was observed in the second month. However, an improvement of body weight was noted in the last month in all groups (Fig. 4). The repeated oral administration of PhHm extract did not cause any significant changes in the weight of the organs in the treated groups compared to the control group. However, a significant increase in the relative mass of the stomach for the group treated with 200 mg/kg was observed (Table 2).

Table 2
Effect of chronic oral administration of PhHm extract on rat's organs weight (g).

	Controls	100 mg/kg	200 mg/kg	300 mg/kg
Liver	7.74 ± 0.61	8.11 ± 1.13 [a]	8.34 ± 1.040 [a]	6.72 ± 0.39 [a]
Heart	0.60 ± 0.05	0.68 ± 0.08 [a]	0.69 ± 0.080 [a]	0.67 ± 0.08 [a]
Kidney	0.65 ± 0.03	0.65 ± 0.07 [a]	0.56 ± 0.04 [a]	0.58 ± 0.03 [a]
Spleen	0.65 ± 0.03	0.60 ± 0.04 [a]	0.56 ± 0.04 [a]	0.60 ± 0.04 [a]
Stomach	0.46 ± 0.03	0.49 ± 0.07[d]	0.54 ± 0.08 [d]	0.46 ± 0.07[c]
Lung	2.53 ± 0.80	3.37 ± 0.52 [a]	7.72 ± 3.34 [a]	4.87 ± 1.25 [a]
Values represent the mean ± SD (N =8). a: not significant (*p>0.05*); c: *p<0.001*; d: *p<0.0001*.				

Changes in the hematopoietic system have greater predictive value for human toxicity. The effect of the chronic oral administration of PhHm extract on the hematologic parameters is presented in Table 3. An increase of the platelet numbers was observed in the treated groups in the 60 and 90 days of the treatment compared to the control group (*p < 0.01*). All the other

parameters (hematocrit, WBC and RBC) remained within the normal limits along the period of the treatment. The biochemical parameters of the treated and control rats are presented in figure 5. The renal profile of the rats was assessed based on the value of urea and creatinine. The chronic oral administration of PhHm extract caused crucial decrease of urea in the treated animals ($p < 0.01$) compared to the control until the last day of treatment. A decrease in creatinine was also observed in the treated groups compared to the control group in the 1st ($p < 0.01$) and 3rd ($p < 0.01$) month of the study and no changes were observed in the 2nd month of the study ($p>0.05$). The liver enzymatic profile of the rats was evaluated based on the level of three liver enzymes named alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase. The statistical analysis indicated that there were no significant differences ($p>0.05$) in the levels of ALP and AST in the treated animals and the control group. The ALT activity was not affected during the 1st and 2nd month of study, nonetheless, an increase of its activity was noted in the treated group ($p<0.01$) compared to the control in the 3rd month (Fig. 6).

Table 3
Effect of PhHm extract on hematological parameters in female Wistar rats treated for 90 days.

	Days	Control	100 mg/kg	200 mg/kg	300 mg/kg
WBC (10^9 L)	30	9.40 ± 0.50	9.20 ± 1.15 [a]	11.30 ± 1.17 [a]	9.30 ± 4.20 [a]
	60	7.52 ± 1.17	7.8 ± 5.29 [a]	9.28 ± 2.77 [a]	7.7 ± 5.01 [a]
	90	5.56 ± 0.68	5.89 ± 0.71 [a]	7.24 ± 0.85 [a]	5.44 ± 0.46 [a]
Platelets	30	220.2 ± 115	690 ± 120 [a]	940.3 ± 70.5 [a]	800.3 ± 172 [a]
(10^9 L)	60	308.7 ± 218	783 ± 274 [a]	811.6 ± 162 [a]	746 ± 524 [a]
	90	875.6 ± 163	731 ± 58.22 [b]	739 ± 69.53 [b]	699 ± 35.92 [b]
RBC (10^{12} L)	30	4.94 ± 0.05	5.11 ± 1.03 [a]	6.88 ± 0.04 [a]	4.01 ± 1.36 [a]
	60	5.59 ± 1.25	6.452± 1.7 [a]	7.08 ± 0.22 [a]	6.05 ± 2.91 [a]
	90	8.29 ± 0.09	7.77 ± 0.31 [a]	8.68 ± 0.05 [a]	9.97 ± 0.46 [a]
Hematocrit (%)	30	29.39 ± 1.52	21.88 ± 1.30 [a]	40.33 ± 0.55 [a]	24.97 ± 1.30 [a]
	60	35.22 ± 7.05	39.42 ± 10.4 [a]	43.92 ± 0.73 [a]	36.3 ± 17.50 [a]
	90	43.56 ± 0.96	44.34 ± 2.44 [a]	46.74 ± 0.64 [a]	53.64 ± 0.63 [a]
Hemoglobin (g/dL)	30	13.34 ± 0.13	13.4 ± 0.1 [a]	18.04 ± 0.05 [a]	11.28 ± 2.33 [a]
	60	14.22 ± 2.52	15.62 ± 3.99 [a]	17.2 ± 0.4 [a]	13.96 ± 6.48 [a]
	90	14.46 ± 0.21	14.46 ± 0.81 [a]	14.92 ± 0.10 [a]	24.60 ± 7.75 [a]

Values represent the mean ± SEM (n =8/group). a: not significant ($p>0.05$); b: p<0.01

Histopathological evaluation of the vital organs, liver and kidney displayed no relevant macroscopic or histological changes in animals that received the PhHm extract (100, 200 and 300 mg/kg). Observation of histological sections of the kidney and the liver in treated rats showed a normal architecture and no evidence of lesion (adequate glomeruli and normal tubules) compared to the control group. However, minimal vascular congestion on liver tissues around the vessels of some treated rats (100, 200 and 300 mg / kg) and inflammatory infiltrate between the renal tubes were observed in animals treated with 100 mg / kg **(Fig. 7)**. Minimal blood congestion was also observed on kidney histological analysis in 100, 200 and 300 mg/kg, and sinusoidal dilation for the higher dose (300 mg/kg) **(Fig. 7)**.

3.6. *In vivo* antioxidant activity

The *in vivo* analysis of the plasma antioxidant capacity of treated and control groups was estimated using DPPH assays. The antioxidant capacity of plasma (ACP) against the DPPH radical was expressed as a percentage (Table 4). The improvement in CAP was significant in treated groups compared to the control group. The results obtained showed that the Vit C (100 mg/kg) and PhHm extract (300 mg/kg) improved the CAP of the rats with an I% of 27.19 ± 0.75% and 29.01 ± 1.33%, respectively. Administration of 300 mg/kg of PhHm extract significantly ($p < 0.01$) increased CAT activity compared to the control group; it had an activity of 11.80 ± 1.24 µmole/min/mg of protein (Table 4). Following the analysis of the Table 4, we notice that the administration of the PhHm extract caused a significant, increase of GSH compared to the control group. The present study revealed low levels of MDA in the liver tissue of treated groups with Vit C and with the extract (100, 200 and 300 mg/kg) (Table 4).

Table 4
Estimation of CAT activity (µmole/min/mg of protein), GSH (nmole/g of tissue) and MDA (nmole/g of tissue) in liver of rat.

Groups	Control group	100 mg/kg BW (Vit C)	100 mg/kg BW	200 mg/kg BW	300 mg/kg BW
CAT	9.07 ± 0.97	4.24 ± 0.57^{b}	5.35 ± 0.79^{a}	8.98 ± 1.60 ^{a}	11.80 ± 1.24 ^{a}
GSH	25.25 ± 0.98	39.45 ± 4.18 ^{c}	12.07 ± 0.96 ^{b}	16.70 ± 0.91 ^{a}	35.18 ± 4.90 ^{a}
MDA	27.95 ± 0.81	22.55 ± 0.45 ^{a}	22.03 ± 0.81 ^{a}	15.65 ± 1.17 ^{c}	15.51 ± 0.28 ^{c}
DPPH (%)	26.28 ± 0.32	27.19 ± 0.75 ^{a}	17.73 ± 1.70 ^{d}	19.81 ± 1.91^{c}	29.01 ± 1.33 ^{a}
Values represent the mean ± SEM (n =8/group). a : not significant ($p>0.05$); b : p<0.01; c : p<0.001; d : p<0.0001					

4. Discussion

In this study, we investigated some biological properties and the safety profile of PhHm extract; a plant growing in the semi-arid region of Algeria. The extraction was performed using methanol 85 % and the yield of extraction of the PhHm extract (24.8 ± 0.74%) was similar to the one found by Rezzagui et al. (2020) (20.18 %). The extraction yield appears to be influenced by the several factors such as the polarity of the solvent (Medjeldi et al., 2018). The hydromethanolic extract presented a higher total phenolic, flavonoids and flavonols content. Atrooz et al. (2018) reported that the total phenolic contents of the PhHm extract were less than those found in our study. Previous reports indicated that the most suitable solvent for the extraction of phenolic compounds from the medicinal and aromatic plants is methanol (Djidel et al., 2013).

Peganum harmala L. can be an interesting source of bioactive compounds such as polyphenols.

Antioxidants are believed to play an important role in preventing the formation of free radicals in the body. The results showed that PhHm extract was able to decolorize the stable, purple-colored DPPH radical into yellow-colored DPPH-H in a concentration dependent way. The IC_{50} values of the PhHm extract were similar to values reported by Baghiani et al. (2012). It was found that the phenolic compounds in the *P. harmala* L. seeds were the major source of its antioxidant activity. Hydromethanolic seed extract of *P. harmala* L. containing higher amount of lipophilic compounds, this is in accordance with the findings by Baghiani et al. (2012). The chelating capacity of our extract is probably due to the presence of antioxidant molecules capable of complexing with ferrous ions. The chelating power of our extracts is important, it helps inhibit peroxidation.

The criteria of cytotoxicity for a crude extracts, established by the U.S. National Cancer Institue (NCI) in the preliminary assays (Ellithey et al., 2014), report that the extracts with an IC50≤ 30µg/ml are strongly cytotoxic. The PhHm extract induced a significant decrease in cell viability and presented lower IC_{50} (0.031 ± 0.007 mg/mL) for HeLa cell, so we can consider it as an

active and potential source of cytotoxic molecules toward HeLa cancer cells. Atrooz et al. (2018) showed that after treatment with methanolic extract of *P. harmala* L. the apoptotic process of the cancer cell line increased compared to the control. These cytotoxic products may be able to play a vital role in treating selected cancers by working in synergy with conventional chemotherapeutic drugs (Yaacob et al., 2010).

The safety of any herbal preparation is a serious issue that needs to be highlighted and any claimed bioactivity or medicinal benefits of plant extracts needs to be coupled with a safety analysis (Jordan et al., 2010). According to Hodge and Sterner (1992), PhHm seed's extract (50< LD_{50} <500 mg/kg B.W), can be classified in the category of moderately toxic chemicals. The LD_{50} values of the PhHm extract were lower than the ones found by Rezzagui A (2020) (DL_{50} : 2860 mg/kg). On the other hand, they were almost similar to what was found by Gseyra (2006) (DL_{50} : 290 mg/kg). The extract may have retarded the animal's growth, as the decrease in body weight may reflect an abnormal growth of mice caused by the altered metabolism of the mice (Mukinda and Eagles, 2010). Organ weight is an important indication of the pathological and physiological status of animals (Benjamin et al., 2020). This implied

that PhHm at 800 mg/kg did not pose any pathological threat to vital organs like liver, kidneys, spleen and heart.

AST, ALT and ALP, are important biomarkers of hepatic functions (Zouhal et al. 2020). Consequently, the alteration of ALT and ALP activity may be an indication of the hepatotoxic potential of PhHm extract at 800 mg/kg. Creatinine and urea were used as biomarkers of renal dysfunction (Lameire et al., 2005).In the present study, a significant ($p<0.01$) dose-dependent decrease in creatinine was observed, which may indicate muscle wastage (Perrone et al., 1992) and may also depend on external factors as rich protein food or decreased protein catabolism. Such congestion could be due to the vasoconstricting action of PhHm extract on the wall of blood vessels (Ashley, 2004). The leukocyte infiltration present in liver of groups treated (500 and 800 mg/kg) could be the result of a liver inflammation.

In the chronic toxicity study, normal rats were treated orally with the PhHm extract for 90 consecutive days in order to observe its chronic effects. The results indicated that the repeated oral administration of PhHm extract did not disrupt the normal growth of mice and did not cause any significant changes in the weight of the organs. Such results indicated no toxic effect in the treated groups ($p > 0.01$). The hematopoietic system is one of the most sensitive targets to toxic compounds and an important index of the physiological and pathological state of humans and animals (Diallo et al., 2010). The increase in urea and plasma creatinine indicates impaired ability of the kidneys to filter waste products from the blood and to excrete them in the urine. On the other hand, the elevated serum urea levels could be due to destruction of red blood cells (Wasnaa, 2010).

The effect of the treatment with the PhHm extract on the state of rat global defense towards free radicals was evaluated. Plasma from control was able to scavenge the DPPH radical. This can be explained by the endogenous antioxidants present in the plasma such as uric acid, albumin, bilirubin and reduced glutathione (GSH).Therefore, the increase in plasma antioxidant capacity may be attributed to high levels of exogenous antioxidants such as flavonoids and phenolic compounds, acquired following treatment with the extract of *P. harmala* L. The observed results are in agreement with Andallu et al. (2011) that suggested that the overall antioxidant capacity of plasma (CAP) results from the synergistic action of endogenous antioxidants and various phytochemical molecules from the administered extracts.

CAT is a ubiquitous enzyme that catalyzes the breakdown of hydrogen peroxide (H_2O_2) a reactive species of oxygen, which is a toxic product of normal aerobic metabolism and the production of pathogenic ROS. Antioxidant enzymes can be inactivated by lipid peroxides and by the ROS produced (Ilari et al., 2020) ; SOD is inhibited by hydrogen peroxide (H_2O_2), while glutathione peroxidase (GPX) and catalase (CAT) are inhibited by an excess of superoxide radical ($O_2^{\bullet}$) (Malheiros et al., 2020). Therefore, supplementing with exogenous antioxidants (polyphenols, flavonoids and others) may play a role in preventing the build-up of ROS and therefore improve the activity of antioxidant enzymes.

Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in cell membranes that cause destruction and damage leading to changes in membrane permeability and fluidity (El-Megharbel et al., 2014) and generates a number of degradation products, resulting in oxidative stress (Najeeb et al., 2012). Hence, the measurement of lipid peroxidation is an important indicator in the assessment of antioxidant potential. The MDA, which is a product of lipid peroxidation, is also one of the most

frequently used biomarkers to evaluate the antioxidant activity *in vivo* (Reddy et al., 2017). This result suggests that *P. harmala* L. hydromethanolic extract is able to prevent the peroxidation of hepatic lipids by improving the antioxidant status of liver.

5. Conclusion

The present study demonstrated that PhHm extract is fairly toxic with LD_{50}: 500 mg/kg B.W. Moreover, daily administration of the PhHm extract at doses ranged from 100 to 300 mg/kg B.W resulted in alteration of the histology of liver and kidney. However, the use of extract of *P. harmala* L. is safe at doses lower than 100 mg/kg B.W. In terms of antioxidant activity, it is concluded that PhHm extract possessed potent antioxidant activity and could be utilized as new natural antioxidant in food and therapeutics. The results of the present investigation suggest that PhHm seed extract has significant antitumor activity, and could be an important basis for the design and synthesis of new antitumor drugs.

Declarations

Acknowledgement

Thanks are due to Engineer of the Higher Institute of Biotechnology of Sfax, Tunisia for her skilful technical assistance. We would like to thank Dr. Boudjlel Amel, Faculty of nature and life, Msila University, Algeria, for the voucher specimen of the plant material used in this study. My sincere thanks to Dr BENBACHA Faycal, Head of the anapathology laboratory of the Public-Hospital Establishment (HPE) of Bordj Bou-Arreridj, for interpreting our histological sections. I would also like to thank Dr Taibi Siham and Dr Ghorieb Isamil, doctors in the anapathology laboratory at Bouzidi Lakhdar Hospital, Bordj Bou Arreridj for providing all the facilities for making histological slides and interpreting them.

References

1. **Abu Sitta KH, Shomah MS, Salhab AS**. Hepatotoxicity of "*Teucrium Polium*" L Tea: Supporting Evidence in Mice Models. *National Herbalists Association of Australia. 21*(4), 106-109. 2009.
2. **Andallu B, Shankaran M, Ullagaddi R, Allagadda VK**. Efficacy of curry (Murraya koenigii) leaves in scavenging free radicals in vitro and controlling oxidative stress *in vivo*. *Biomedicine and Preventive Nutrition*, *1*(4), 263-267. 2011. DOI : https://doi.org/10.1016/j.bionut.2011.06.005
3. **Antonious GF, Kochhar TS, Jarret RL, Syder JC.** Antioxidants in Hot Pepper: Variation Among Accessions. *Journal of Environmental Science and Health, Part B. 41*(7), 1237-1243. 2006. DOI: https://doi.org/10.1080/03601230600857114
4. **Ashley C**. Renal failure: how drugs can damage the kidney. Royal *Free Hamstead NHS Trust, 11*, 48–53. 2004.
5. **Atrooz OM, Joanna W, Beata FP, Ibrahim AR, Soub M, Mohammad HA**.. Antiproliferative, antioxidant, and antibacterial activities of crude plant extracts of *Asphodeline lutea* L. and *Peganum harmala* L. *Pharmaceutical research, 11*, 148-167. 2018. DOI: https://doi.org/10.20959/wjpr201811-12522
6. **Baghiani A, Djarmouni M, Boumerfeg S, Hayet T, Charef N, Khennouf S, Arrar L**.. Xantine oxidase inhibition and antioxidant effects of *Peganum harmala* seeds extracts. *European Journal of Medicinal Plants, 2*, 42-56. 2012. DOI: https://doi.org/10.9734/EJMP/2012/898
7. **Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Gazin JC, Inkas M, Uycky M, Gazin M**. Oxygen species scavenging activity of phenolic extract from howthorn fresh plant organs and pharmaceutical preparation. *Arzneim Forsch, 46*, 1086–1094. 1996.
8. **BenjaminZG, Ngbolua KN, Tshibangu DST, Memvanga PB, Tshilanda DD, Matondo A, Kilembe JT, Lebwaze BM, Nachtergael A, Mpiana PT, Duez P.** *In vivo* Evaluation and in silico Prediction of the Toxicity of Drepanoalpha® hard capsules. 2020. DOI: https://doi.org/10.1101/2020.12.03.411124
9. **Burits M, Bucar F**. Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research, 14*(5), 323-328. 2000. DOI: https://doi.org/10.1002/1099-1573(200008)14:5<323::AID-PTR621>3.0.CO;2-Q

10. **Claiborne A**. Catalase activity. In: Greenwald, R.A. (Ed.), CRC handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, FL, 283–284. 1986.
11. **Cuendet M, Hostettmann K, Dyatmiko W, Potterat O**. Iridoid glucosides with free radical scavenging propreties from Fagraea blumei. *Helvitica Chimica Acta, 80* (4), 1144–1152. 1997. DOI: https://doi.org/10.1002/hlca.19970800411
12. **Dapkevicius A, Venskutonis R, Van Beek TA, Linssen JPH**. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *Journal of the Science of Food and Agriculture, 77*(1), 140-146. 1998. DOI: https://doi.org/10.1002/(SICI)1097-0010(199805)77:1<140::AID-JSFA18>3.0.CO;2-K
13. **Decker EA, Welch B**. Role of Ferritin as a Lipid Oxidation Catalyst in Muscle Food. *Journal of Agricultural and Food Chemistry*, *38*(3), 674-677. 1990. DOI: https://doi.org/10.1021/jf00093a019
14. **Diallo A, Eklu-Gadegkeku K, Agbonon A, Aklikokou K, Creppy EE, Gbeassor M.** Acute and Subchronic (28-Day) Oral Toxicity Studies of Hydroalcolic Extract of Lanneakerstingii Engl. And K. Krause (Anacardiaceae) Stem Bark. *Journal of Pharmacology and Toxicology, 5*, 343–349. 2010. DOI: https://doi.org/ 10.4314/tjpr.v9i5.61059
15. **Djidel S, Khennouf S, Ameni D, Baghiani A, Arrar L, Charef N**. Antioxidant proprieties of *Pistacia lentiscus* L. Leaves extracts. *Pharmacognosy Communications, 3*(2), 28-34. 2013. DOI: https://doi.org/10.5530/pc.2013.2.7
16. **Ellithey MS, Lall N, Hussein AA, Meyer D**. Cytotoxic and HIV-1 enzyme inhibitory activities of Red Sea marine organisms. *BMC Complementary and Alternative Medicine, 14*(1), 77. 2014. DOI: https://doi.org/10.1186/1472-6882-14-77
17. **Ellman GL**. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics, 82*, 70–77. 1959.
18. **El-Megharbel SM, Hamza RZ, Refat MS**. Preparation, spectroscopic, thermal, antihepatotoxicity, hematological parameters and liver antioxidant capacity characterizations of Cd(II), Hg(II), and Pb(II) mononuclear complexes of paracetamol anti-inflammatory drug. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 131*, 534-44. 2014. DOI: https://doi.org/10.1016/j.saa.2014.04.108
19. **Gseyra N**. Etude phytochimique de deux espèces pastorales : *Artemisia herba alba* Asso. et *Peganum harmala* L. et l'évolution de la toxicité de *Peganum harmala* pendant son cycle phénologique. Université Mohamed Premier, Faculte des sciences Oujda. 122-124. 2006.
20. **Hodge HC, Sterner JH**. Tabulation of toxicity classes. *American Industrial Hygiene Association Quarterly, 10*(4), 93-98. 1992. DOI : https://doi.org/10.1080/00968204909344159
21. **Ilari S, Giancotti LA, Lauro F, Gliozzi M, Malafoglia V, Palma1 E, Tafani M, Russo MA, Tomino C, Fini M, Salvemini D, Mollace V, and Muscoli C**. Natural Antioxidant Control of Neuropathic Pain—Exploring the Role of MitochondrialSIRT3 Pathway. *Antioxidants, 9*(11), 1-20. 2020. DOI: https://doi.org/10.3390/antiox9111103
22. **Jalali A, Dabaghian F, Zarshenas MM.** Alkaloids of *Peganum harmala*: Anticancer Biomarkers with Promising Outcomes. *Current Pharmaceutical Design, 27*(2), 185-196(12). 2021. DOI : https://doi.org/10.2174/1381612826666201125103941
23. **Jordan SA, Cunningham DG, Marles RJ**. Assessment of herbal medicinal products: Challenges, and opportunities to increase the knowledge base for safety assessment. *Toxicology and Applied Pharmacology, 243*(2), 198-216. 2010. DOI: https://doi.org/10.1016/j.taap.2009.12.005
24. **Kosalec I, Bakmaz M, Pepeljnjak S, Vladimir-Knežević S**. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharmaceutica, 54*, 65-72. 2004.
25. **Lameire N, Van Biesen W, Vanholder R**. Acute renal failure. *The Lancet, 365* (9457), 417-430. 2005. DOI: https://doi.org/10.1016/S0140-6736(05)17831-3
26. **Le K, Chiu F, Ng K**. Identification and quantification of antioxidants in Fructus lycii. *Food Chemistry, 105*(1), 353-363. 2007. DOI : https://doi.org/10.1016/j.foodchem.2006.11.063
27. **Li H Bin, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y**. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry. 102*(3), 771-776. 2007. DOI: https://doi.org/10.1016/j.foodchem.2006.06.022
28. **Çelik ND, Ger Akarsu F, Ozkan S, Özdemir AÖ, Aykac Ö & Memmedova F**. Herbal teas can be harmful: a case of *Peganum harmala* induced coma in a patient with Parkinson's disease. *Neurocase The Neural Basis of Cognition, 27*(2), 117-119.

2021. DOI: https://doi.org/10.1080/13554794.2021.1876092

29. **Malheiros RSP, Gonçalves FCM, Brito FAL, Zsögön A, & Ribeiro DM**. Selenomethionine induces oxidative stress and modifies growth in rice (Oryza sativa L.) seedlings through effects on hormone biosynthesis and primary metabolism. *Ecotoxicology and Environmental Safety, 189*, 109942. 2020. DOI: https://doi.org/10.1016/j.ecoenv.2019.109942
30. **Markham, K.R**. (1982). Techniques of flavonoid identification. London: Academic press.
31. **Martey ON, Armah G, Okine LKN**. Absence of organ specific toxicity rats treated with Tonica, an aqueous herbal haematinic preparation. *African Journal of Traditional, Complementary and Alternative Medicines, 7*(3), 231–240. 2010. DOI: https://doi.org/10.4314/ajtcam.v7i3.54781
32. **Medjeldi S, Bouslama L, Benabdallah A, Essid R, Haou S, Elkahoui S.** Biological activities, and phytocompounds of northwest Algeria *Ajuga iva* (L) extracts: Partial identification of theantibacterial fraction. *Microbial Pathogenesis, 121*, 173-178. 2018. DOI: https://doi.org/10.1016/j.micpath.2018.05.022
33. **Mosmann T**. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*, 55–63. 1983. DOI : https://doi.org/10.1016/0022-1759(83)90303-4
34. **Mukinda JT, Eagles PFK**. Acute and sub-chronic oral toxicity profiles of the aqueous extract of Polygala fruticosa in female mice and rats. *Journal of Ethnopharmacology, 128*(1), 236-240. 2010. DOI : https://doi.org/10.1016/j.jep.2010.01.022
35. **Najeeb Q, Bhaskar N, Masood I, Wadhwa S, Kaur H, Ishaq S**. Malondialdehyde (MDA) superoxide dismutase (SOD) levels-distinguishing parameters between benign malignant pleural effusions. *Free Radicals and Antioxidants, 2*(2), 8–11. 2012. DOI: https://doi.org/10.5530/ax.2012.2.2.2
36. **OECD (Organization for Economic Co-operation and Development)**. Guidelines for the testing of chemicals. *Acute Oral. Toxicity Proced, 425*, 427. 2008.
37. **Niazi S, Enferadi ST and Ghaderitabar H.** Variation of Main Alkaloids and Fatty Acids among Five Natural Populations of *Peganum harmala* L. from Iran. *Journal of Medicinal Plants and By-products*, 1: 45-52. 2021. DOI : https://doi.org/10.22092/JMPB.2020.128202.1151
38. **OECD.** Test no. 407: Repeated dose 28-day oral toxicity study in rodents. *Drug and Chemical Toxicology, 34*(1), 77–84. 2008b.
39. **Okhawa H, Ohishi N, Yagi K**. Assay of lipid peroxides in animal tissue by thiobarbituric reaction. *Analytical Biochemistry, 95*, 351–358. 1979. DOI: https://doi.org/10.1016/0003-2697(79)90738-3
40. **Perrone RD, Madias NE. and Levey AS**. Serum Creatinine as an Index of Renal Function: New Insights into Old Concepts. *Clinical Chemistry, 38*(10), 1933-1953. 1992. DOI : https://doi.org/10.1093/clinchem/38.10.1933
41. **Qiu K, Li Z, Li C, Huang H, Zhu W**. Protective effect of total glycosides from lily on H2O2-induced H9C2 cells mitochondrial damage and characterization of the chemical profiles by UHPLC-LTQ-Orbitrap-MSn. *Journal of Functional Foods, 71*, 104036. 2020. DOI : https://doi.org/10.1016/j.jff.2020.104036
42. **Rasekh HR, Yazdanpanah H, Hosseinzadeh L, Bazmohammadi N, Kamalinejad M**. Acute and Subchronic Toxicity of Teucrium polium Total Extract in Rats. *Iranian Journal of Pharmaceutical Research, 4*(4), 245-249. 2010.
43. **Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C**. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine. 26(9), 1231-1237. 1999. DOI: https://doi.org/10.1016/S0891-5849(98)00315-3
44. **Reddy UA, Prabhakar PV. and Mahboob M**. Biomarkers of oxidative stress for *in vivo* assessment of toxicological effects of iron oxide nanoparticles. *Saudi Journal Biological Science, 24*, 1172-1180. 2017. DOI: https://doi.org/10.1016/j.sjbs.2015.09.029
45. **Rezzagui A, Merghem M, Derafa I, Dahamna S.** Acute and Sub-acute Toxic Effects of Algerian *Peganum harmala* L. Crud Extract. *Journal of Drug Delivery & Therapeutics, 10*(2), 115-121. 2020. DOI: https://doi.org/10.22270/jddt.v10i2.3920
46. **Wasnaa H.** Histological and functional study of white male mice testes and kidney treated with Tecriumpolium aqueous extract. *Journal of Enginneering and Technology Management, 28*(20), 6149-6153. 2010.
47. **Waynforth HB.** Experimental and surgical technique in the rat. London, Axademic Press. 17-68. 1980.

48. **Yaacob NS, Hamzah N, Nik Mohamed Kamal NN, Zainal Abidin SA, Lai CS, Navaratnam V, Norazmi MN**. Anticancer activity of a sub- fraction of dichloromethane extract of *Strobilanthes crispus* on human breast and prostate cancer cells *in vitro*. *BMC Complementary Medicine and Alternative Medicine, 10*(1), 42. 2010. DOI: https://doi.org/10.1186/1472-6882-10-42

49. **Yeung AWK, Tzvetkov NT, Georgieva MG, Ognyanov IV, Kordos K, Jóźwik A, Kühl T, Perry G, Petralia MC, Mazzon E, and Atanasov AG.** Reactive oxygen species (ROS) and their impact in neurodegenerative diseases: literature landscape analysis. *Antioxidants & Redox Signaling, 34*(5). 2020. DOI : https://doi.org/10.1089/ars.2019.7952

50. **Yu M, Gouvinhas I, Rocha J, Barros AIRNA**. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Scientific Reports, 11,* 10041. 2021. DOI: https://doi.org/10.1038/s41598-021-89437-4

51. **Zouhal H, Bagheri R, Ashtary-Larky D, Wong A, Triki R, Hackney AC, Laher I, Abderrahman AB**. Effects of Ramadan intermittent fasting on inflammatory and biochemical biomarkers in males with obesity. *Physiology & Behavior, 225, 113090.* 2020. DOI: https://doi.org/10.1016/j.physbeh.2020.113090

Figures

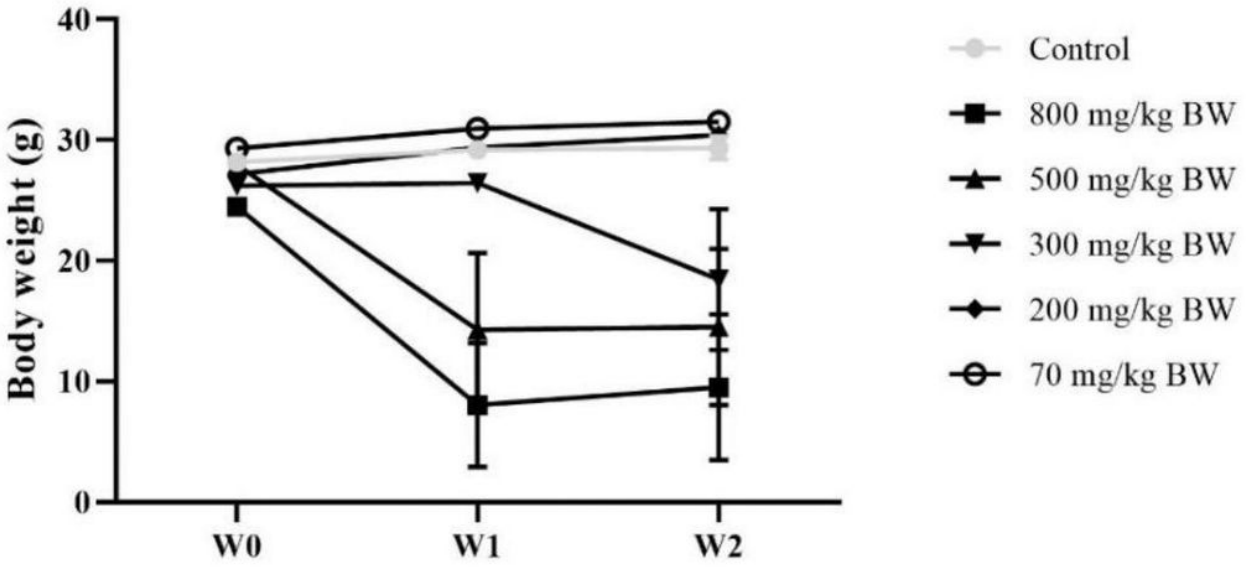

Figure 1

Body weight of mice treated orally with PhHm extract. Values expressed as mean ± SEM, N=8 animals/group.

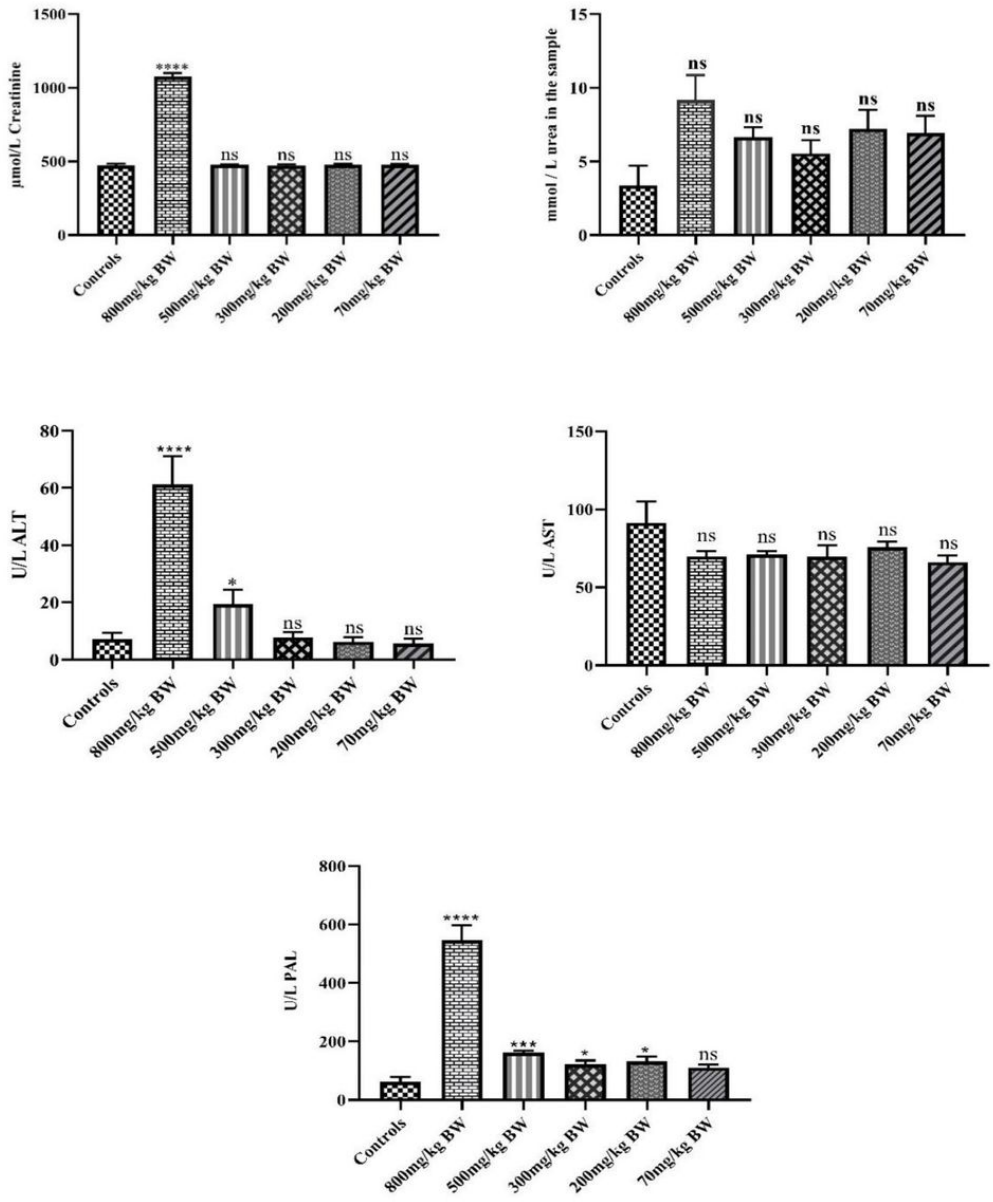

Figure 2

Biochemical parameters of control and mice treated with PhHm extract measured during the acute toxicity. Values were expressed as mean ± SEM (N = 8). ns: no significant, *: p<0.01,**: p < 0.001, ****: p < 0.0001.

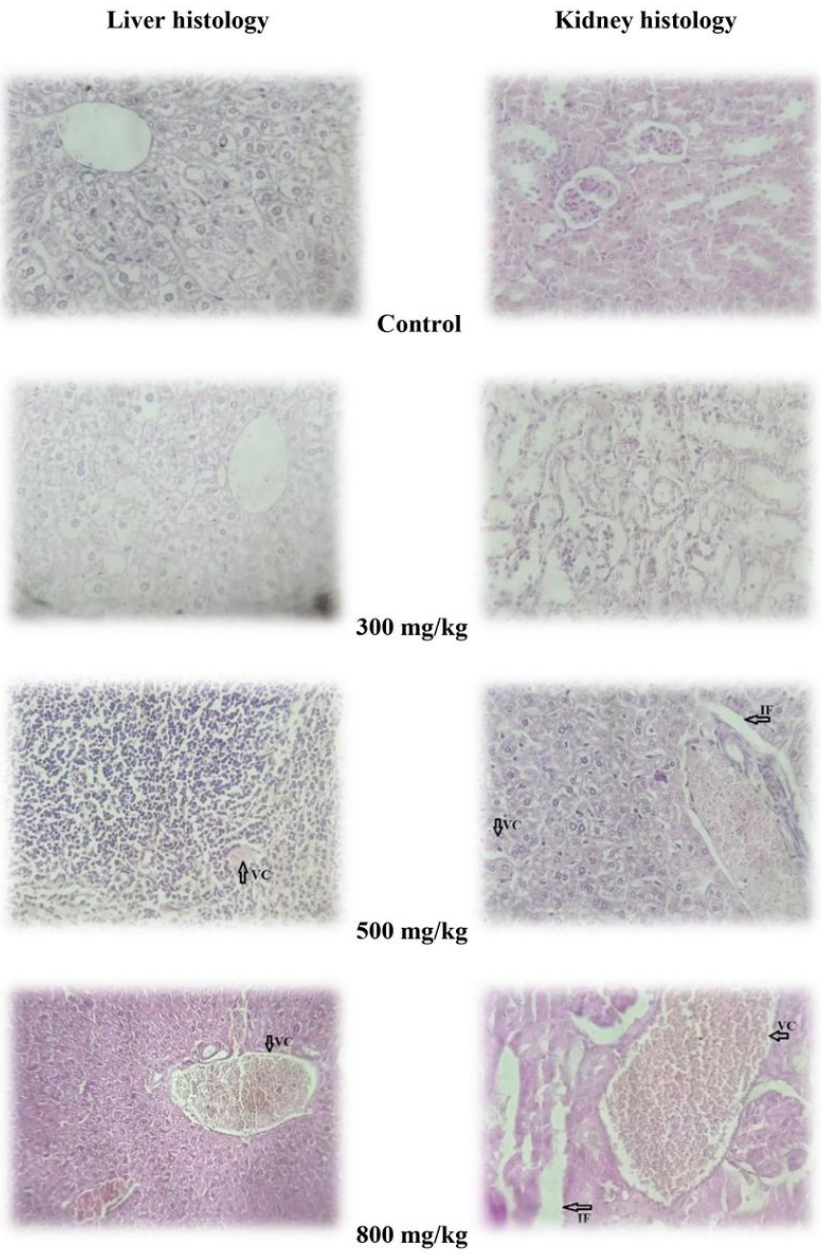

Figure 3

Histopathological analysis of the liver and kidneys of animals treated with PhHm extract. in acute toxicity (x 40). CV: Vascular Congestion, IF: inflammatory infiltrate.

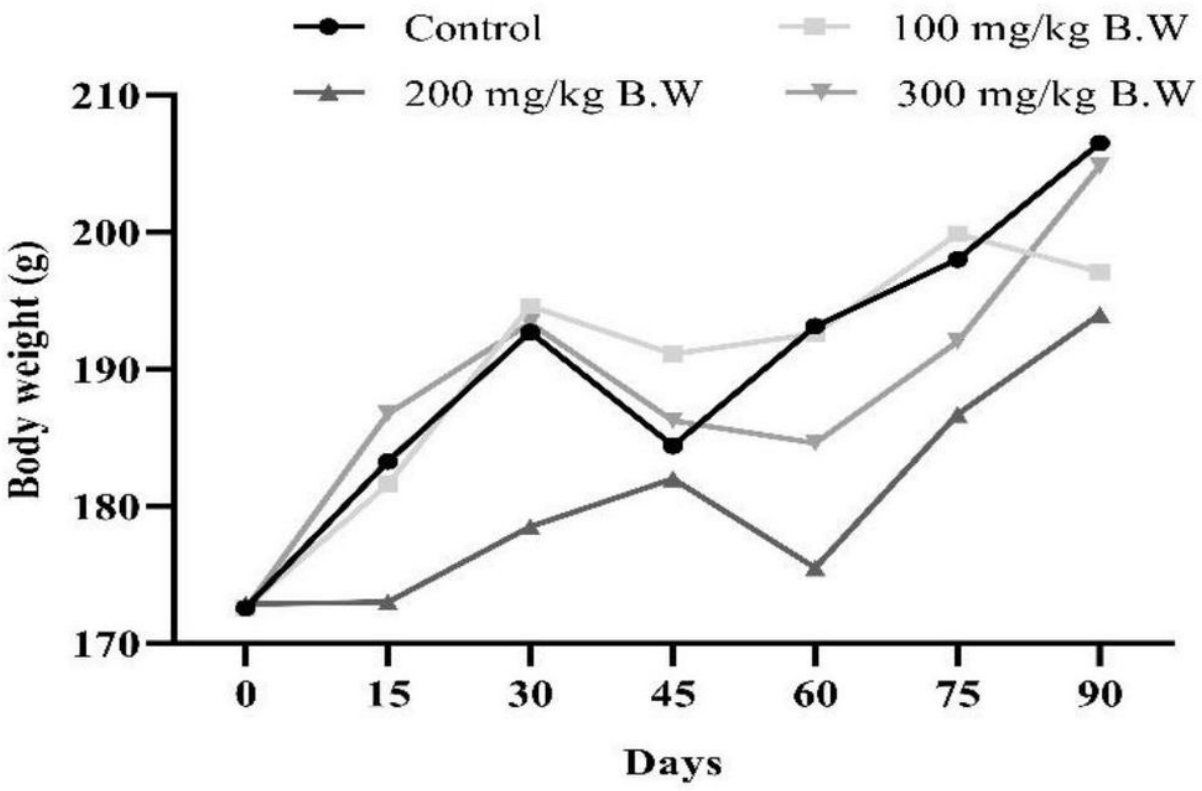

Figure 4

The effect of chronic oral administration of PhHm extract on the body weight of rats. Data are expressed as mean ± SEM.

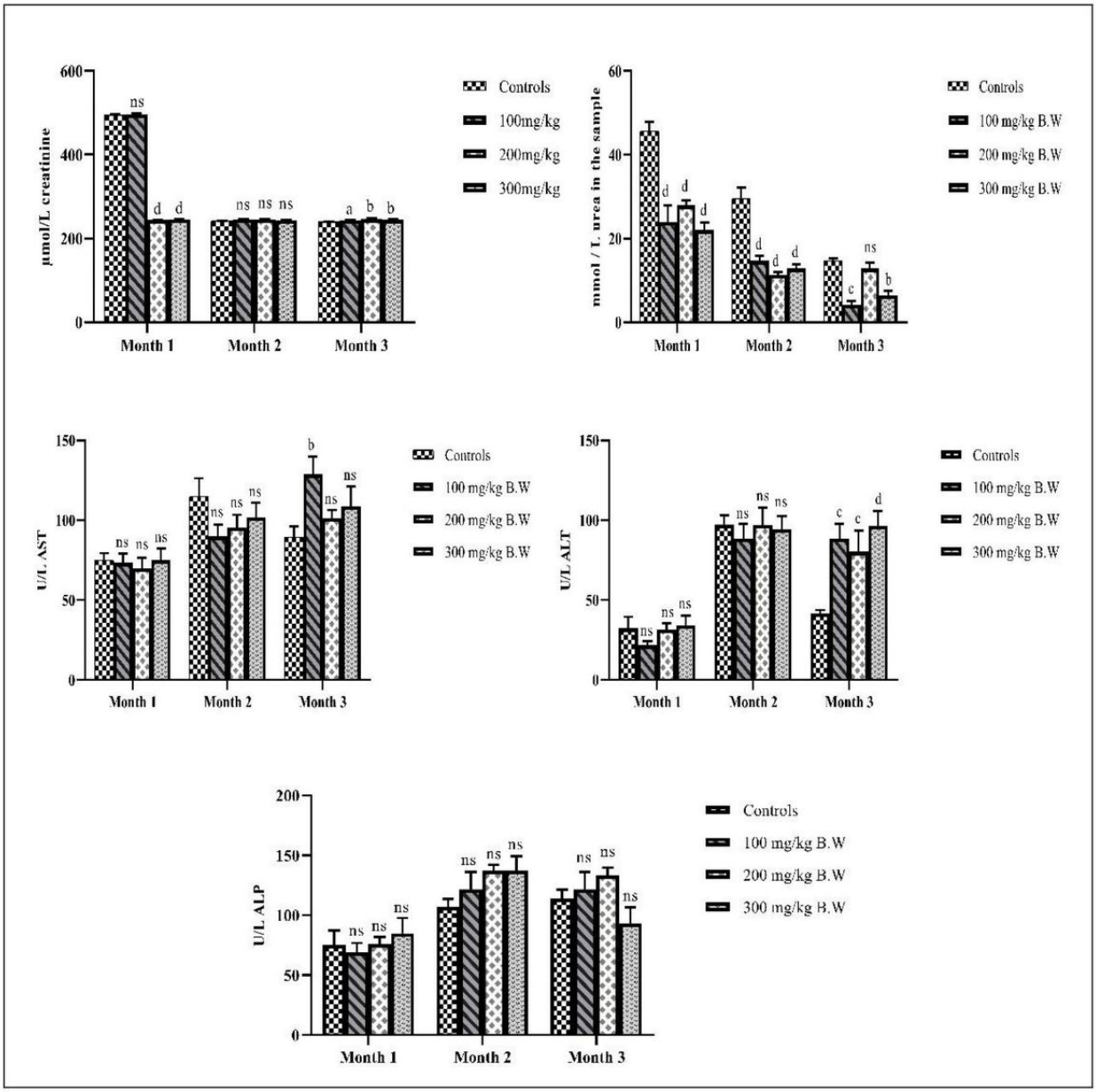

Figure 5

Biochemical parameters of control and treated groups with PhHm extract measured during the chronic toxicity. Values were expressed as mean ± SEM (N = 8). ns: no significant, a : $p < 0.01$, b: $p < 0.001$, c and d: $p < 0.0001$.

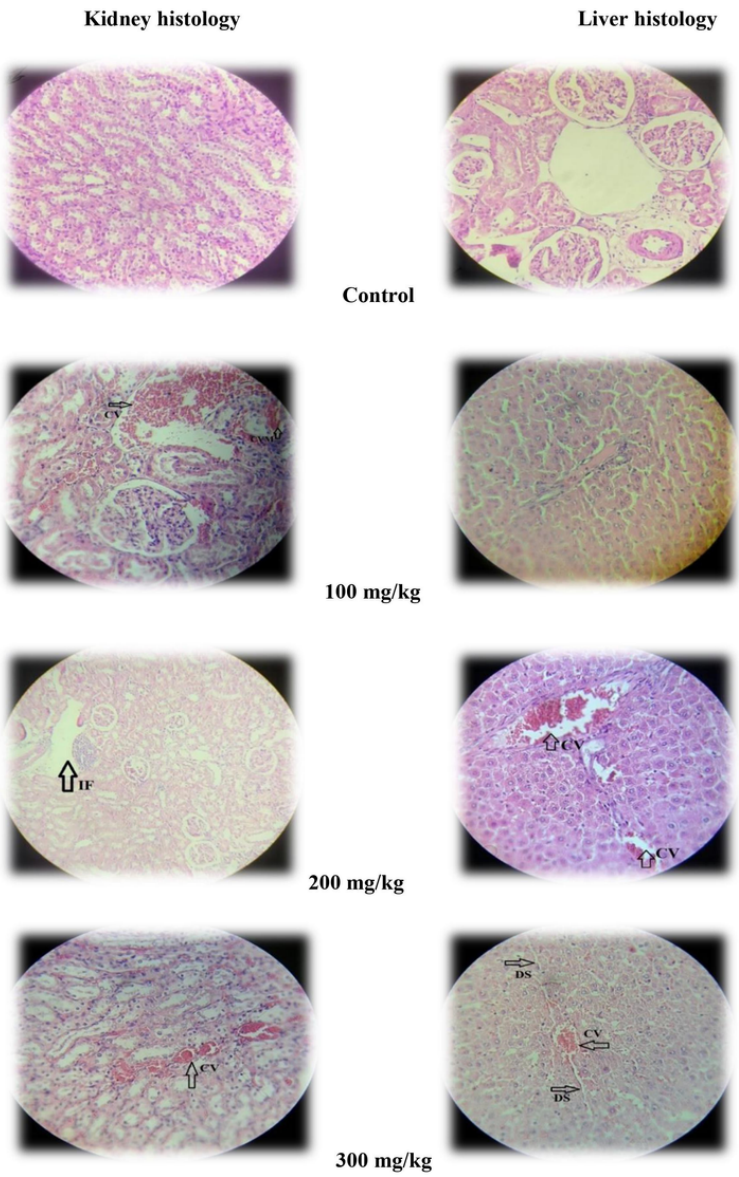

Figure 6

Renal and hepatic histological cuts of treated animals and control group in chronic toxicity (x 40). CV: Vascular Congestion, IF: inflammatory infiltrate. DS: sinusoidal dilatation. A: control group, B: animals treated with 100 mg/kg, C: animals treated with 200 mg/kg, D: animals treated with 300 mg/kg.