Int. J. Morphol.,
44(1):165-174, 2026.

Morphofunctional Changes in Liver and Kidney Tissues Following Acute and Subchronic Exposure to *Berberis hispanica* Bark Extract in Mice

Cambios Morfofuncionales en Tejidos Hepáticos y Renales Tras la Exposición Aguda y Subcrónica al extracto de Corteza de *Berberis hispanica* en Ratones

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KACI, S.; SMAIL, L.; BOUDJLIDAA, A.; KACIMI, G.; BELKEBIR, A.; HAMLAT, N. & BOUGUERRA, S. A. Morphofunctional changes in liver and kidney tissues following acute and subchronic exposure to *Berberis hispanica* bark extract in mice. *Int. J. Morphol., 44(1)*:165-174, 2026.

SUMMARY: Several anticancer agents currently used in clinical settings are derived from plants, such as vinblastine and vincristine from *Catharanthus roseus*, and paclitaxel from *Taxus brevifolia*. In this context, the present study investigates the acute and subchronic toxicity of *Berberis hispanica*, a medicinal plant traditionally used in North Africa, particularly in the Tikjda region, for its therapeutic properties. The aim was to assess its potential toxic effects on liver and kidney function in female *Mus musculus* mice. The aqueous extract was prepared from the dried root bark through maceration in water, followed by filtration and concentration. Mice were randomly divided into groups and received daily oral doses of 0, 75, 375, 750, or 1500 mg/kg of extract for six weeks. Acute toxicity was evaluated based on behavioral changes and mortality within 24 hours. Subchronic toxicity was assessed through biochemical analyses of serum liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase) and renal markers (urea, creatinine, albumin), as well as histopathological examination of liver and kidney tissues. No mortality or significant behavioral changes were observed following acute administration. Subchronic exposure to the highest dose (1500 mg/kg) resulted in a 33 % mortality rate and mild histological alterations. Biochemical markers showed limited variations compared to controls, suggesting a low level of hepatorenal toxicity. These findings indicate that the aqueous extract of *Berberis hispanica* exhibits low acute and subchronic toxicity in mice, supporting its potential for therapeutic use, pending further pharmacological and toxicological evaluation.

KEY WORDS: *Berberis hispanica***; Toxicity; Liver function; Kidney function.**

INTRODUCTION

Traditional medicine has long played a crucial role in the treatment and prevention of various human diseases, particularly in regions where access to conventional healthcare is limited. Approximately 80 % of the world's population still relies on plant-based remedies for their primary healthcare needs (WHO, 2019). Among these, medicinal plants with a wide spectrum of biological activities have garnered increased attention in recent years, both from the scientific community and the general public, as alternatives or complements to synthetic drugs (Yuan *et al*., 2016). *Berberis* species, notably *Berberis hispanica*, belong to the Berberidaceae family and are traditionally used in various cultures for their therapeutic properties. *B. hispanica* is a deciduous shrub widely distributed in the Mediterranean basin, particularly in North Africa and the Iberian Peninsula. This plant has been historically employed in folk medicine for the management of gastrointestinal disorders, fever, and kidney stones, and is known for its antimicrobial and anti-inflammatory properties (Belwal *et al*., 2020). The pharmacological potential of *Berberis* is largely attributed to its alkaloid content, especially berberine, a bioactive isoquinoline compound. Berberine exhibits a wide array of biological effects, including antioxidant, hepatoprotective, nephroprotective, and cardioprotective activities (Cervello *et al*., 2024). These properties make it a strong candidate for further investigation in toxicity and efficacy studies, particularly regarding the safety profile of aqueous extracts used in traditional practices. Given the growing interest in validating the safety of herbal products,

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Received: 2025-07-31 Accepted: 2025-09-20

this study aims to evaluate the acute and subchronic toxicity of an aqueous extract of *Berberis hispanica* bark (EATBh) in mice. We assessed its effects on biochemical markers of hepatic and renal function, as well as histomorphological changes in liver and kidney tissues, to determine the potential risks associated with its prolonged use.

MATERIAL AND METHOD

Preparation of the Total Aqueous Extract

The aqueous extract was prepared following the method of (Sasidharan *et al*., 2011) with slight modifications. The root bark of *Berberis hispanica* was ground into a fine powder using an electric grinder, then suspended in distilled water for 12 hours on a thermostatically controlled magnetic stirrer at 95?°C. The resulting mixture was filtered, and the filtrate was dried at 90 °C for 24 h. The final extract was stored at 4 °C.

The extraction yield was calculated as follows: *REaq = (M´/ M) x 100*. Where *REaq*: productivity of aqueous extract, M´: mass of the aqueous extract obtained in gram, M: mass of dry plant material in grams.

Phytochemical study

Determination of Total Polyphenols and Flavonoids. The total polyphenol content was determined using the method of Singleton & Rossi (1965). The Folin–Ciocalteu reagent is a yellow acidic solution composed of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). During the oxidation of phenolic compounds, the reagent is reduced to a mixture of blue-colored oxides of tungsten and molybdenum. The intensity of the resulting blue coloration is proportional to the polyphenol content in the extract and is measured spectrophotometrically at 685 nm. Flavonoids have the ability to form complexes with metal ions through chelation. Their content was determined using the aluminum chloride ($AlCl_3$) colorimetric method. This method is based on the chelating properties of Al^{3+} ions with flavonoids, leading to the formation of either labile or stable complexes. The absorbance of these complexes is measured using a spectrophotometer at 440 nm.

Antioxidant Activity of the Total Aqueous Extract of *Berberis hispanica*. The antioxidant activity was evaluated using two methods:

-The Phosphomolybdenum Method. This method is based on the reduction of Mo (VI), present as molybdate ions (MoO_4^{-2}), to Mo(V) in the presence of the extract. The reduced molybdenum then forms a green phosphate/Mo(V) complex under acidic pH conditions (Prieto *et al*., 1999). The total antioxidant capacity (TAC) is expressed in milligrams of ascorbic acid equivalents per gram of fresh matter (mg AAE/g FM).

- The DPPH Method (2,2-diphenyl-1-picrylhydrazyl) DPPH is a stable free radical that exhibits a deep violet color at room temperature. The antioxidant activity is measured by the ability of the extractto reduce DPPH• to DPPH-H, resulting in a color change from violet to yellow. The degree of discoloration indicates the scavenging capacity of the antioxidant compounds present in the extract (Brand-Williams *et al*., 1995).

Qualitative and Quantitative Analysis by HPLC of Berberine, the Main Alkaloid of *Berberis*, and the Total Aqueous Extract of *Berberis hispanica*. The HPLC system used for analytical control is equipped with a Shimadzu SPD-20 UV/Vis detector. The system includes the following components: four isocratic Perkin-Elmer (USA) pumps with a quaternary gradient capability, a Rheodyne injection valve fitted with a 20 µL sample loop, and a Hypersil BDS RP-C18 column (silica particle size: 5 µm; length: 250 mm; internal diameter: 4.6 mm). The mobile phase consists of a solution of trifluoroacetic acid (0.1 %) and acetonitrile in a 62:38 (v/v) ratio. After membrane filtration through a 0.45 µm filter and degassing, the mobile phase is delivered at a flow rate of 1 mL/min. Detection is carried out at a wavelength of 350 nm. Both the total extract and the berberine standard are dissolved in HPLC-grade methanol. The concentration of alkaloids is determined by extrapolation from a previously established calibration curve using commercial alkaloid standards of known concentrations.

Experimental Animals

Ethical Approval. The present study was approved by the Institutional Animal Care and Use Committee of the National Administration of Algerian Higher Education and Scientific Research (DGRSDT; *https://www.dgrsdt.dz*) and the University of Bab Ezzouar (Algiers, Algeria). The permit number for this research project is F00220110048. The study was conducted in accordance with Executive Decree No. 10-90, supplementing Executive Decree No. 04-82 of the Algerian government, which defines the terms and conditions for animal welfare in experimental animal facilities.

Diet. The standard laboratory diet used was formulated according to the specifications of the National Livestock Feed Office (ONAB) (*https://www.onabnutrition.dz*). The average daily intake per rat was 20 g, corresponding to approximately 9 kilocalories.

Animals. The study was conducted on female *Mus musculus* mice maintained under standard laboratory conditions in the animal facility of the Faculty of Biological Sciences at the University of Sciences and Technology Houari Boumediene (USTHB). Animals were housed in a controlled environment with a 12-hour light/dark cycle, temperature of 22 ± 2 °C, and relative humidity of 50 - 60 %. They were provided with a standard diet formulated by ONAB (Office National des Aliments de Bétail), the composition of which is detailed in Table I, and had free access to food and water *ad libitum*.

A total of 24 young, healthy, nulliparous, and non-pregnant female mice (considered more sensitive than males according to OECD (Organisation for Economic Co-operation and Development), 2008, and not prone to pro-erectile effects) weighing 20 ± 2 g were randomly assigned to six groups: control group (C), and five treatment groups. Mice were acclimatized for approximately one week and fasted for four hours prior to extract administration. The test substance was administered orally using an esophageal gavage probe.

For the sub-chronic toxicity study, mice in the following groups received the extract daily for six weeks: group 1: 0 mg/Kg; group 2: 75 mg/kg; group 3: 375 mg/kg; group 4: 750 mg/kg; Group 5: 1500 mg/kg.

A maximum volume of 0.4 mL per day was administered, in accordance with OECD guidelines, to ensure the dose did not exceed 1 mL per 100 g of body weight. For the acute toxicity study, a single dose of 1500 mg/kg was administered. The animals were observed individually, with close monitoring during the first 4 hours post-administration, followed by regular checks over a 24-hour period.

In the sub-chronic toxicity study, the observation period lasted six weeks. All animals were monitored at least twice daily for signs of morbidity and/or mortality.

At the end of the experimental period, blood samples were collected from the retro-orbital sinus using dry tubes. Due to the limited volume of blood per mouse, samples from each group were pooled. After centrifugation at 3000 rpm, the resulting sera were stored at -20 °C until biochemical analysis.

Assessment of Acute and Sub-Chronic Toxicity. The acute toxicity of *Berberis hispanica* was evaluated in mice according to the OECD Guidelines. The LD_{50} (lethal dose 50) refers to the dose of a substance required to cause the death of 50 % of an animal population under specific experimental conditions (Rates, 2001).

$LD_{50} = LD_{100} - \Sigma ab / n'$. Where n′ = Average number of animals per group; a = Mean number of deaths between two dose levels; b = Difference between two successive doses.

Due to the absence of mortality following the administration of the different doses, the LD_{50} could not be determined.

The sub-chronic toxicity study was based on the repeated oral administration of the test substance over an extended period (a single dose level, daily, for 90 days), in accordance with OECD guidelines.

Biochemical Analysis. The assessment of biochemical markers related to liver and kidney function was performed using a COBAS 2000 automated biochemical analyzer.

Histological Study. This study included both macroscopic and microscopic examinations of the liver and kidney tissues of the experimental animals. Following autopsy, the entire liver was excised immediately. Macroscopic examination was qualitative and focused on the external characteristics of the liver, including color, consistency, and texture. For microscopic analysis, representative tissue samples from the liver and kidneys were collected from each animal. The samples were fixed in paraformaldehyde. Two types of histological staining were performed: Masson's trichrome for connective tissue and fibrosis assessment

RESULTS

Chemical Test Results

Phytochemical Screening. A positive reaction with sulfuric acid indicates the presence of saponosides (Table I). Positive reactions with cyanidin and aluminum chloride suggest the presence of flavonoids. The positive results with ferric

Table I. Qualitative phytochemical analysis of the various compounds in *Berberis hispanica.*

	Compound Type	Observation / Reaction	Interpretation
Heterosides	Saponosides	(+) Reaction with sulfuric acid (I=333,33)	Present
	Flavonoids	(+) Reaction with cyanidin	Present
	Tannins	(+) Reaction with ferric chloride	Present
	Catechins	(-) Reaction with hydrochloric butanol	Absent
	Gallic tannins	(+) Reaction with sodium acetate	Present
	Alkaloids	(+) Reaction with Bouchardat / Dragendorff / Mayer reagents	Present

chloride and sodium acetate indicate the presence of tannins and specifically gallic tannins. Finally, a positive reaction with the Bouchardat, Dragendorff, and Mayer reagents confirms the presence of alkaloids.

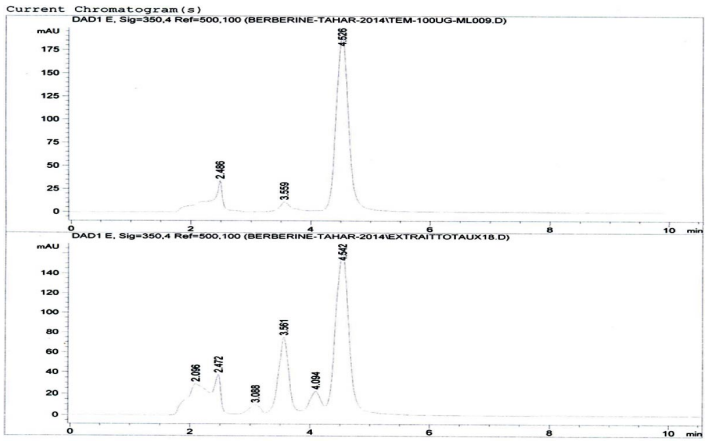


Fig. 1. Chromatogram of the standard berberine and the berberine detected in the aqueous extract of *Berberis hispanica*, showing respective retention times of 4.526 and 4.542 minutes. HPLC conditions for berberine detection: column: C18 silica column (5 µm particle size, 250 mm length, 4.6 mm internal diameter); mobile phase: 0.1% trifluoroacetic acid and acetonitrile (62:38, v/v); flow rate: 1.0 mL/min; detection wavelength: 350 nm

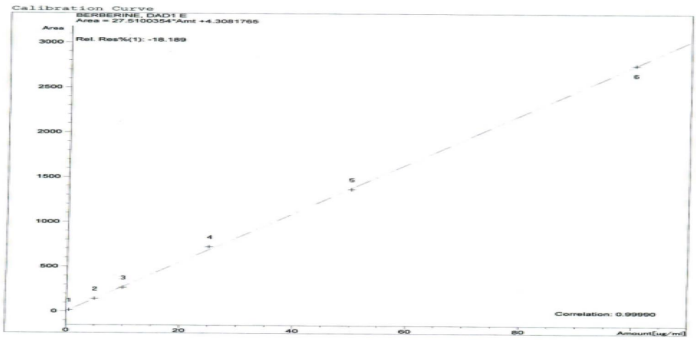


Fig. 2. Calibration Curve for Berberine Quantification. Calibration curve illustrating the linear relationship between peak area and berberine concentration. The regression equation is: Area = 27.51004 × [Berberine] (µg/mL) + 4.30818, with a coefficient of determination R^2 = 0.99990.

HPLC Analysis of the Total Aqueous Extract of *Berberis hispanica* (Bh)

High-performance liquid chromatography (HPLC) analysis of the total aqueous extract of *Berberis hispanica* revealed a prominent peak corresponding to berberine (Fig. 1). This peak exhibited a retention time of 4.542 minutes, closely matching that of the internal standard, berberine, which eluted at 4.526 minutes under the same chromatographic conditions. The concentration of berberine in the extract was determined to be 89.41 µg/mL, based on the standard calibration curve (Fig. 2). This value represents approximately 8 % (w/v) of the total aqueous extract. Additional peaks with distinct retention times suggest the presence of other phytochemical constituents in the extract, which may correspond to various secondary metabolites naturally present in *Berberis hispanica*.

Effects of Acute and Subchronic Toxicity of *Berberis hispanica* (Bh) in Mice

The behavioral and neurological assessments following a single oral dose of 1500 mg/kg of *Berberis hispanica* aqueous extract are summarized in Table II. Under acute toxicity conditions, no mortality was observed, rendering LD_{50} estimation unnecessary. Treated mice displayed normal mobility, regular stool consistency, and normal tail posture. No signs of aggressiveness were recorded, and animals remained alert and responsive throughout the observation period. Under subchronic exposure revealed notable toxicity at higher doses: of five animals treated, two died (40 %). Motor activity increased at moderate doses (375 and 750 mg/kg) but significantly decreased at 1500 mg/kg. Aggressiveness increased at higher doses, whereas alertness and vigilance declined, especially at the highest dose.

Table II. Behavioral and Toxicological Signs in Mice Treated with *Berberis hispanica* Extract.

	Control	0 mg/kg	75 mg/kg	375 mg/kg	750 mg/kg	1500 mg/kg	Acute
Number of animals	5	5	5	5	5	5	5
Locomotor activity	N	N	N	↑	↑	↓	N
Aggressiveness	N	N	N	A	A	↑	A
Stool consistency	N	N	N	N	N	N	N
Piloerection	A	A	A	A	A	A	A
Tail posture	N	N	N	N	N	N	N
Vigilance	+	+	+	+	+	–	+
Number of deaths	0	0	0	0	0	2	0

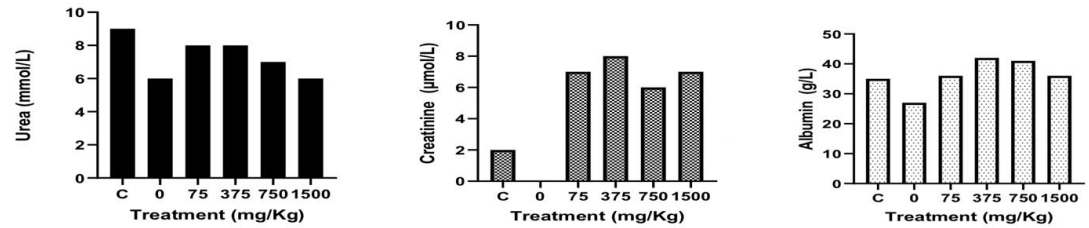
Biochemical Analysis Results

Subchronic Toxicity

-Urea, Creatinine, and Albumin levels. A dose-dependent decrease in serum urea levels was observed across all treated groups, with the most substantial reductions recorded in the 0 mg/kg group (33.3 %) and at the highest dose of 1500 mg/kg (27.7 %). This reduction may be indicative of impaired protein catabolism, potentially reflecting a disruption in nitrogen metabolism (Fig. 3). In contrast, serum creatinine levels increased significantly in all treated groups except the 0 mg/kg group, with a peak increase of 300 % at 375 mg/kg. Serum albumin concentrations remained relatively stable, although moderate increases were noted at 375 mg/kg (20 %) and 750 mg/kg (17.1 %). These fluctuations may indicate an adaptive physiological response or a transient alteration in glomerular permeability.

- AST, ALT and PAL levels. A reduction in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels was observed at the higher doses (750 and 1500 mg/kg). The most pronounced decrease in AST was recorded at 1500 mg/kg (47.7 %), while ALT levels decreased by -42.9 % at the same dose, suggesting a potential hepatoprotective effect. In contrast, alkaline phosphatase (PAL) activity increased in a dose-dependent manner, peaking at 1500?mg/kg with a 30 % elevation, which may indicate biliary stress at higher concentrations (Fig. 4).

(a)

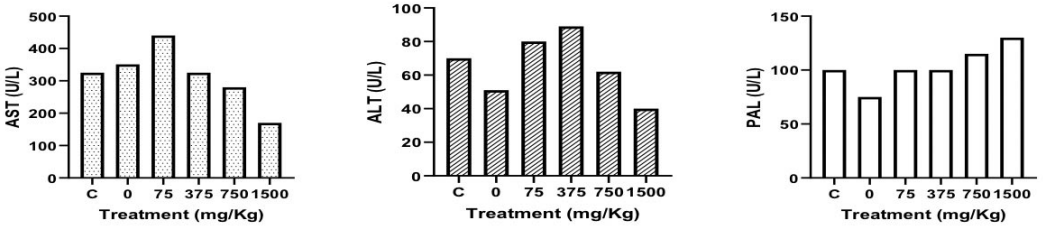


(b)

	Treatment (mg/Kg)				
	0	**75**	**375**	**750**	**1500**
Urea %	-33.3	-11.1	-5.5	-16.6	-27.7
Creatinine %	-100	250	300	200	250
Albumin %	-22.9	2.9	20	17.1	2.9

Fig. 3. Evaluation of serum renal markers in mice after 6 weeks of daily oral administration of *Berberis hispanica* aqueous extract (EATBh) at doses of 0, 75, 375, 750, and 1500 mg/kg. (a) Graphical representation of serum levels of urea (mmol/L), creatinine (µmol/L), and albumin (g/L). (b) Percentage change of each marker relative to the control group (c), expressed as a table.

(a)



(b)

	Treatment (mg/Kg)				
	0	**75**	**375**	**750**	**1500**
AST %	8	35.8	0	-13.8	-47.7
ALT %	-27.1	14.3	27.1	-11.4	-42.9
PAL %	-25	0	0	15	30

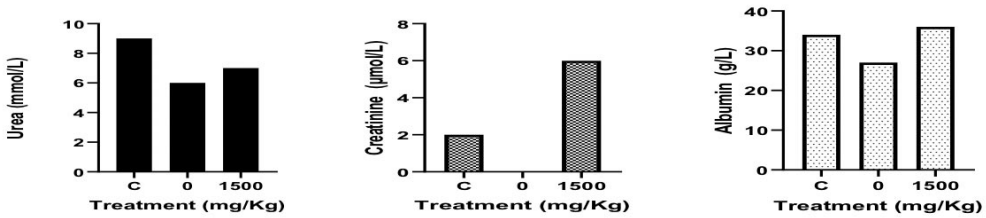
Fig. 4. Evaluation of serum hepatic markers in mice after 6 weeks of daily oral administration of *Berberis hispanica* aqueous extract (EATBh) at doses of 0, 75, 375, 750, and 1500 mg/kg. (a) Graphical representation of serum levels of AST (U/L), ALT(U/L), and PAL (U/L). (b) Percentage change of each marker relative to the control group (C), expressed as a table.

Acute Toxicity

- Urea, Creatinine, and Albumin levels. The results indicate a decrease in serum urea levels in group (0 mg/kg), followed by an increase (22.2 %) in the group receiving a single high dose of 1500 mg/kg (Fig. 5). Creatinine concentrations exhibited marked alterations, with a complete reduction in the group (0 mg/kg) and an increase (200 %) in the EATBh-treated group, suggesting possible renal stress at high doses. Serum albumin levels remained relatively stable.

-AST, ALT and PAL levels. A reduction in AST (35 %) and a moderate decrease in ALT (7 %) were observed in mice treated with 1500 mg/kg, compared to the 0 mg/Kg group and control group (Fig. 6). Conversely, PAL levels increased markedly (129 %) in the treated group, suggesting potential hepatobiliary stress effects associated with high-dose EATBh exposure.

(a)

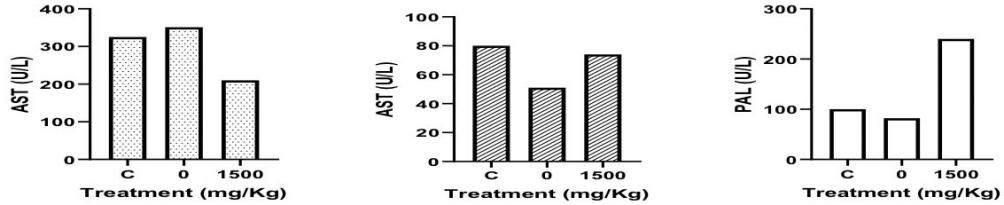


(b)

	Treatment (mg/Kg)	
	0	**1500**
Urea %	-33.3	22.2
Creatinine %	-100	200
Albumin %	-20.6	5.9

Fig. 5. Evaluation of serum renal markers in mice after acute exposure to 1500?mg/kg of *Berberis hispanica* total aqueous extract (EATBh). (a) Graphical representation of serum levels of urea (mmol/L), creatinine (µmol/L), and albumin (g/L). (b) Percentage change of each marker relative to the control group (C), expressed as a table.

(a)



(b)

	Treatment (mg/Kg)	
	0	**1500**
AST%	8	-35.4
ALT%	-36.3	-7.5
PAL%	-18	140

Fig. 6. Evaluation of serum hepatic markers in mice after acute exposure to 1500?mg/kg of *Berberis hispanica* total aqueous extract (EATBh). (a) Graphical representation of serum levels of AST (U/L), ALT(U/L), and PAL (U/L). (b) Percentage change of each marker relative to the control group (C), expressed as a table.

Histological Study

Liver: Histological analysis of liver sections from mice treated with different doses of *Berberis hispanica* aqueous extract (EATBh) revealed dose-dependent morphological changes. In the control group (Fig. 7A), liver architecture appeared normal, with well-organized hepatic cords and intact sinusoids. In the vehicle control group (0 mg/kg, Fig. 7B), the liver structure remained largely preserved, though mild sinusoidal dilation and venous stasis were observed. At 75 mg/kg (Fig. 7C), hepatocytes maintained a polyhedral shape with central nuclei, but slight vascular congestion suggested early adaptive responses. At 375 mg/kg (Fig. 7D), cytoplasmic vacuolization appeared in centrilobular hepatocytes, indicating initial signs of cellular stress. More pronounced alterations were evident at 750 mg/kg (Fig. 7E), including intracytoplasmic vesicles and dilated central veins, consistent with moderate hepatic

injury. Surprisingly, at 1500 mg/kg (Fig. 7F), liver morphology was largely preserved, suggesting potential adaptive or protective mechanisms activated at higher doses.

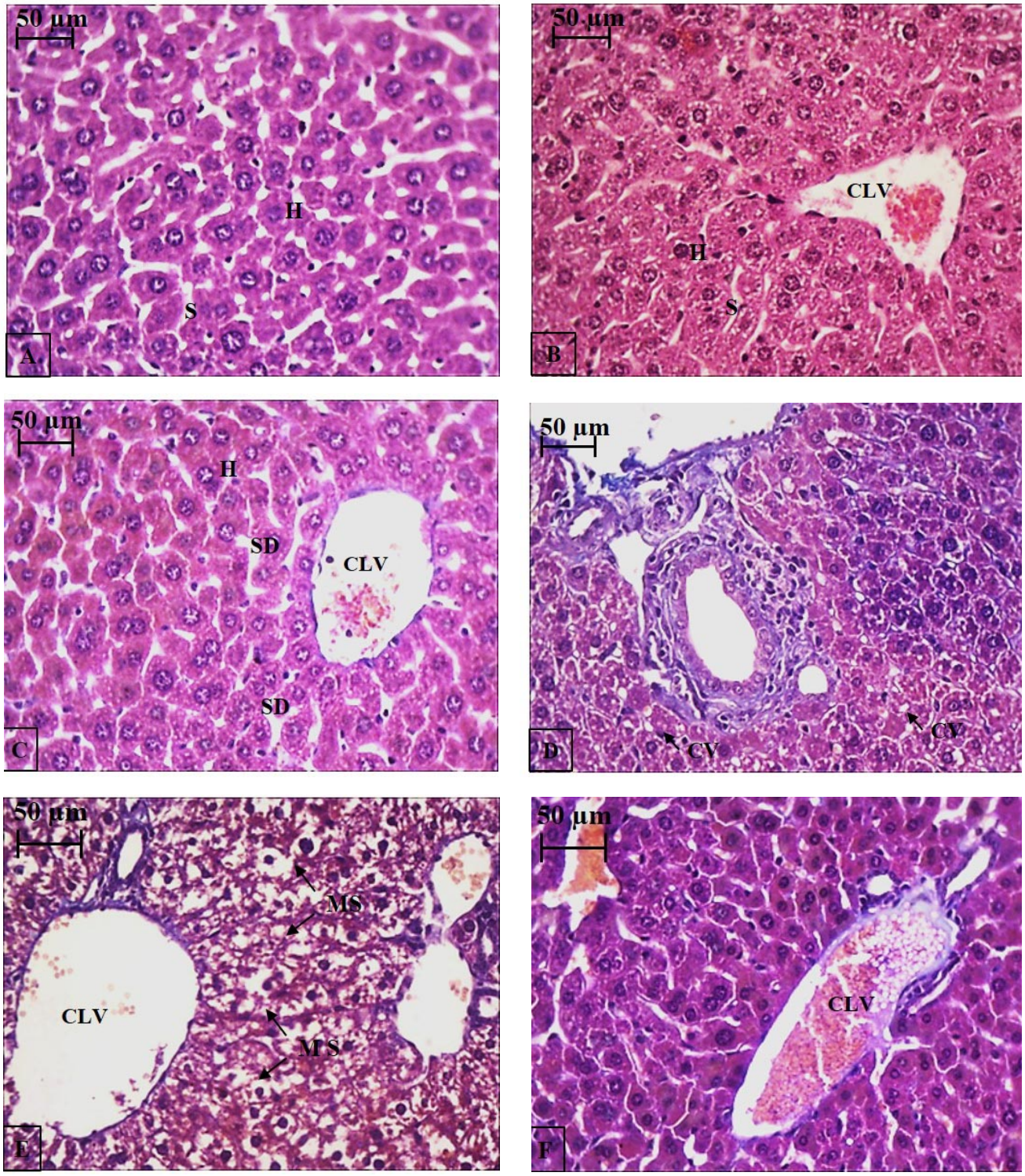


Fig. 7. Representative histological sections of liver tissue stained with Masson's trichrome and observed under light microscopy at 40× magnification (scale bar = 50 µm), from mice treated orally for 6 weeks with different doses of the total aqueous extract of *Berberis hispanica* (EATBh). (A) Control group, (B) (0 mg/kg) group, (C) 75 mg/kg group, (D) 375 mg/kg group, (E) 750 mg/kg group, (F) 1500 mg/kg group, (H) hepatocyte, (S) sinusoid, (SD) sinusoidal dilatation, (CLV) centrilobulair vein, (MS) steatosis microvesiculaire, (CV) cytoplasmic vacuolization.

Renal cortex. Histological examination of the renal cortex demonstrated preserved architecture of glomeruli and tubules, with no major pathological lesions observed at any dose of *Berberis hispanica* extract, suggesting an absence of overt nephrotoxicity under the tested conditions (Fig. 8).

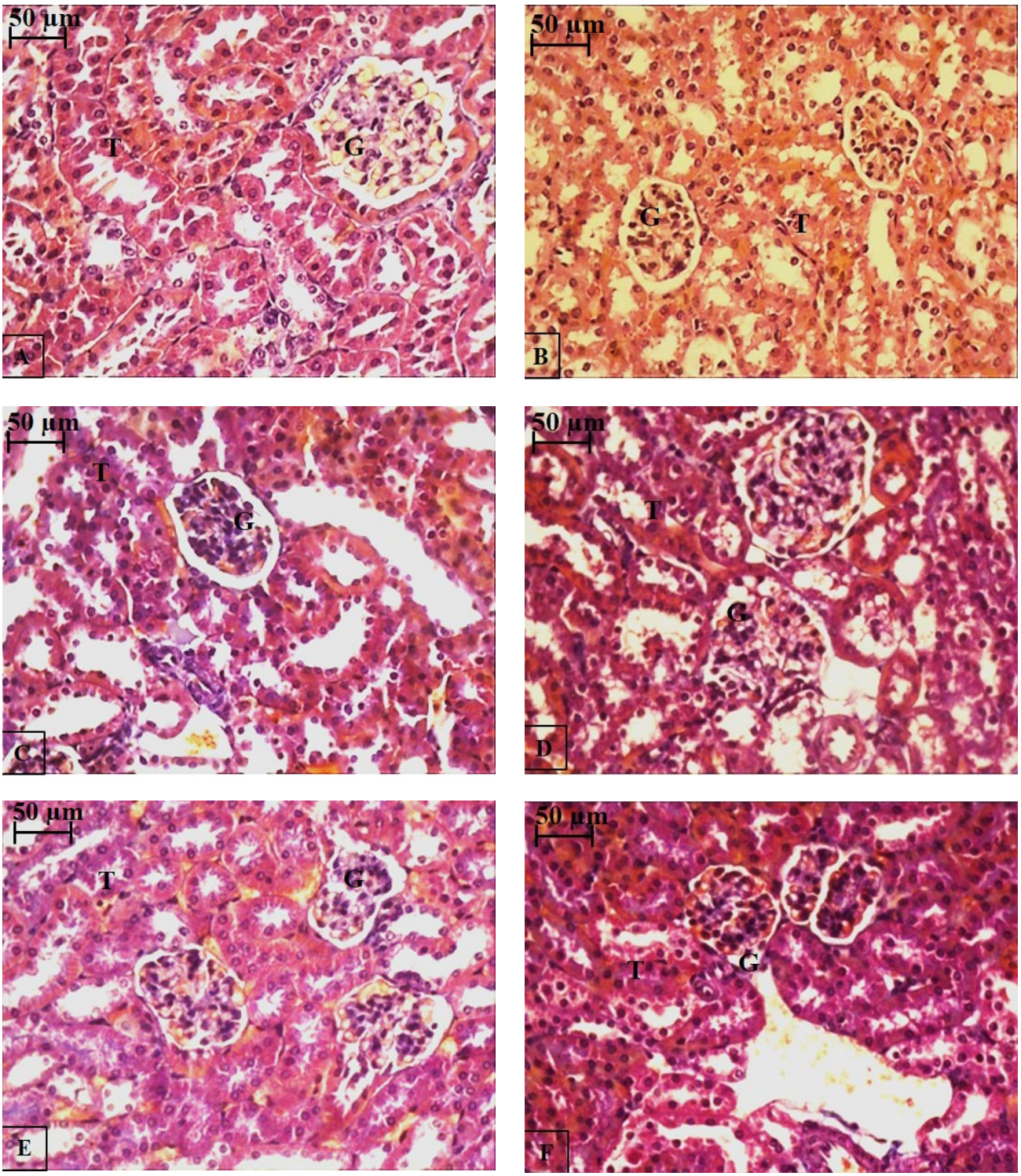


Fig. 8. Representative histological sections of renal cortex tissue stained with Masson's trichrome and observed under light microscopy at 40× magnification (scale bar = 50 µm), from mice treated orally for 6 weeks with different doses of the total aqueous extract of *Berberis hispanica* (EATBh). (A) Control group, (B) (0 mg/kg) group, (C) 75 mg/kg group, (D) 375 mg/kg group, (E) 750 mg/kg group, (F) 1500 mg/kg group, (G) Glomerulus, (T) Tubule.

DISCUSSION

Phytochemical screening of *Berberis hispanica* aqueous extract revealed the presence of several bioactive secondary metabolites, including flavonoids, tannins (notably gallic tannins), saponins, and alkaloids. These findings are consistent with previous studies reporting the richness of *Berberis* species in alkaloids particularly protoberberine derivatives such as berberine as well as polyphenolic compounds and flavonoids (Cushnie & Lamb, 2011). The presence of flavonoids, as indicated by positive reactions with cyanidin and aluminum chloride, supports the potential antioxidant and hepatoprotective properties of the extract (Cushnie & Lamb, 2011). The detection of alkaloids using Bouchardat, Dragendorff, and Mayer reagents confirms the abundance of these compounds, particularly berberine, a well-known bioactive isoquinoline alkaloid with diverse pharmacological properties, including anti-inflammatory, antidiabetic, and anticancer effects (Kulkarni & Dhir, 2010). HPLC analysis further validated these findings by identifying a major peak with a retention time of 4.542 minutes, matching that of the berberine standard (4.526 minutes). The berberine content of the extract was quantified at 89.41 µg/mL, representing approximately 8 % (w/v) of the total extract. Similar berberine concentrations have been reported in *Berberis* vulgaris and other *Berberis* species (Potdar *et al*., 2012). The high berberine concentration reinforces the potential therapeutic applications of *B. hispanica*, although this also raises concerns regarding dose-dependent toxicity, as alkaloids such as berberine can accumulate in tissues and affect mitochondrial function at higher doses (Zhou *et al*., 2016). Acute toxicity testing showed no signs of mortality or behavioral abnormalities up to a single oral dose of 1500?mg/kg, suggesting a high margin of safety and placing the LD?? above this threshold. According to OECD guidelines and the classification proposed by Diezi (1989), substances with an LD?? greater than 2000 mg/kg are considered relatively non-toxic, while those between 500 - 2000 mg/kg are considered mildly toxic. Our results are therefore in line with studies on other *Berberis* species, where aqueous extracts demonstrated low acute toxicity (Imenshahidi & Hosseinzadeh, 2016). However, subchronic exposure revealed dose-dependent signs of toxicity. At the highest dose (1500 mg/kg), 40 % mortality was observed, accompanied by significant behavioral changes such as decreased motor activity, reduced vigilance, and increased aggressiveness. These findings may be attributed to cumulative alkaloid toxicity, consistent with previous studies reporting neurotoxic and hepatotoxic effects of berberine and related compounds upon prolonged exposure. Interestingly, at moderate doses (375 and 750 mg/kg), animals exhibited increased locomotor activity, which could reflect a stimulatory effect on the central nervous system, a phenomenon previously described with certain plant-derived alkaloids. A dose-dependent decrease in urea levels was observed, particularly marked at 1500 mg/kg. Although such a decline might be interpreted as favorable, it may also indicate altered nitrogen metabolism or early hepatic dysfunction (Adedapo *et al*., 2004). Creatinine, a classical marker of glomerular filtration, increased significantly starting from 75 mg/kg, peaking at 375 mg/kg with a 300 % elevation, suggesting functional renal impairment or mild to moderate tubular toxicity (Jung *et al*., 2008). Albumin levels remained relatively stable, indicating preserved glomerular integrity and minimal protein loss (Chevalier, 2005). ALT and AST activities decreased significantly at higher doses, particularly at 1500 mg/kg (AST: -47.7 %, ALT: -42.9 %), potentially indicating a hepatoprotective effect of *Berberis* compounds, especially berberine, known for its antioxidant and anti-inflammatory properties (Chen *et al*., 2021). Conversely, the progressive increase in alkaline phosphatase (ALP) activity suggests potential biliary stimulation or early cholestasis (Kaplowitz, 2001). This mixed biochemical profile has also been observed in studies involving other *Berberis* species (Chen *et al*., 2021). Liver histology revealed moderate alterations starting from 375 mg/kg, including cytoplasmic vacuolization and vascular congestion typical signs of hepatocellular stress. However, the relatively preserved hepatic architecture at 1500 mg/kg may reflect an adaptive response or protective enzyme induction, as previously reported with other plant extracts rich in alkaloids (Hermenean *et al*., 2012). Renal tissue remained largely intact, even at the highest doses, supporting the hypothesis that the observed biochemical changes are functional and reversible, without significant morphological damage. Under acute exposure, the increase in creatinine (+200 %) and ALP (+140 %) at 1500 mg/kg suggests transient hepatorenal stress without severe histological damage, aligning with previous reports on isoquinoline alkaloid toxicity (Forouzanfar *et al*., 2014).

CONCLUSION

In summary, the total aqueous extract of *Berberis hispanica* demonstrates a favorable safety profile in acute exposure, with no lethality or major toxicological manifestations observed at doses up to 1500 mg/kg. However, repeated subchronic administration over six weeks induced dose-dependent biochemical and histopathological alterations, particularly affecting hepatic and, to a lesser extent, renal function. These findings underscore the necessity for cautious use of *Berberis hispanica*, especially in long-term applications.

KACI, S.; SMAIL, L.; BOUDJLIDAA, A.; KACIMI, G.; BELKEBIR, A.; HAMLAT, N. & BOUGUERRA, S. A. Cambios morfofuncionales en tejidos hepáticos y renales tras la exposición aguda y subcrónica al extracto de corteza de *Berberis hispanica* en ratones. *Int. J. Morphol., 44(1)*:165-174, 2026.

RESUMEN: Varios agentes anticancerígenos utilizados actualmente en la práctica clínica se derivan de plantas, como la vinblastina y la vincristina de *Catharanthus roseus*, y el paclitaxel de *Taxus brevifolia*. En este contexto, el presente estudio investiga la toxicidad aguda y subcrónica de *Berberis hispanica*, una planta medicinal tradicionalmente utilizada en el norte de África,en particular en la región de Tikjda, por sus propiedades terapéuticas. El objetivo fue evaluar sus posibles efectos tóxicos sobre la función hepática y renal en ratones hembra *Mus musculus*. El extracto acuoso se preparó a partir de la corteza de la raíz seca mediante maceración en agua, seguida de filtración y concentración. Los ratones se dividieron aleatoriamente en grupos y recibieron dosis orales diarias de 0, 75, 375, 750 o 1500 mg/kg de extracto durante seis semanas. La toxicidad aguda se evaluó con base en los cambios de comportamiento y la mortalidad dentro de las 24 horas. La toxicidad subcrónica se evaluó mediante análisis bioquímicos de enzimas hepáticas séricas (alanina aminotransferasa, aspartato aminotransferasa, fosfatasa alcalina) y marcadores renales (urea, creatinina, albúmina), así como mediante examen histopatológico de los tejidos del hígado y el riñón. No se observó mortalidad ni cambios significativos en el comportamiento después de la administración aguda. La exposición subcrónica a la dosis más alta (1500 mg/kg) resultó en una tasa de mortalidad del 33 % y alteraciones histológicas leves. Los marcadores bioquímicos mostraron variaciones limitadas en comparación con los controles, lo que sugiere un bajo nivel de toxicidad hepatorrenal. Estos hallazgos indican que el extracto acuoso de *Berberis hispanica* presenta baja toxicidad aguda y subcrónica en ratones, lo que respalda su potencial para uso terapéutico, a la espera de una mayor evaluación farmacológica y toxicológica.

PALABRAS CLAVE: *Berberis hispanica*; Toxicidad; Función hepática; Función renal.

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