Research Article

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Acute and repeated dose 60-day oral toxicity assessment of chemically characterized *Berberis hispanica* Boiss. & Reut in Wistar rats

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Abstract: *Berberis hispanica* Boiss. & Reut (*B. hispanica*) belongs to the family Berberidaceae, which is currently used in traditional medicines. This article aimed to study the phytochemical composition and acute and subacute toxicity of *B. hispanica* extract in rats. The phytochemical composition of *B. hispanica* extract was characterized using GC-MS. The acute toxicity was investigated *in vivo* via the oral administration of single doses including 400, 800, 1,000, 1,200, and 1,400 mg/kg for 14 days. The subchronic toxicity was studied through oral administration of 400 mg/kg for 60 days. The findings of the phytochemical

analysis of *B. hispanica* extract showed the presence of various phytochemical compounds. Acute toxicity results revealed serious clinical symptoms and mortalities in rats treated with 800 mg/kg up to a maximum of 1,400 mg/kg. With acute toxicity, subchronic toxicity results showed also serious signs of toxicity including biochemical and histological alterations in animals treated with 1,400 mg/kg. *B. hispanica* extract revealed to be toxic in rats orally treated under both subacute (>400 mg/kg) and subchronic toxicity conditions (400 mg/kg). The outcome of this study serves the society as it provides toxicological evidence of *B. hispanica* used in alternative medicines

Keywords: *Berberis hispanica* Boiss. & Reut, toxicity, biochemical parameters, histopathology, chemical characterization

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1 Introduction

Herbal medicines have been seen as an exhaustive source of therapeutic agents for many years ago. Nowadays, several developed drugs come from natural products or their derivatives [1]. The use of plants in traditional medicines for disease treatment goes back thousands of years ago. About 80% of people around the world use folk medicines for medication purposes. This huge use could be explained by the fact that most people based in developing countries have no access to modern medicine [2]. Moreover, 25% of prescription drugs used in the United States between 1959 and 1980 were derived from the plant kingdom [3], and about 60% of prescriptions in European countries come also directly or indirectly from plants [4]. The use of the traditional herbal practice can be explained by many reasons such as the high cost of advanced medicines, sociocultural practices of users, and the need for controlling resistant pathogens using natural alternative agents [4,5].

Nowadays, modern medicine has focused on natural products as unexhausted sources for drug production.

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Medicinal plants are extremely numerous, and more than 13,000 species of medicinal plants are used as traditional remedies by various cultures around the world [6,7]. The efficiency of medicine is due to secondary metabolites that vary due to environmental changes. Many secondary metabolites like polyphenols are antibiotics in the broad sense because they protect plants from fungi, bacteria, animals, and even other plants [8]. Toxic plants possess toxic substances to humans or animals, which induce serious clinical signs and even can be fatal after being ingested. The efficacy of plants versus diseases cannot exclude their potentially harmful effects on health, and therefore, any natural product should be used with precautions [9].

Berberis hispanica Boiss. & Reut (B. hispanica) is originally from Europe and the British Isles, which was introduced in North America and Mediterranean countries. It has been used as a medicinal plant for more than 2,500 years [10]. Numerous medicinal uses of B. hispanica were reported in the literature, such as analgesic, antimicrobial, antitonic, antiemetic, anti-pyretic, anti-pruritic, antiarrhythmic, anticholinergic, anti-oxidant, antinociceptive, anti-arrhythmic, antijaundice, anticholelithiasis, antileishmaniasis, antihypertension, anticardiac arrhythmias, antidysentery, anti-inflammatory, antipruritic, anticholagogue, and antidigestive disorders [9-14].

The present study was conceptualized to study the phytochemical composition and acute and subchronic toxicity of B. hispanica used in traditional alternative medicine since no previous study has attempted this objective.

2 Materials and methods

2.1 Plant material and extract preparation

B. hispanica was collected in May 2016 from Middle Atlas (Morocco). The botanical authentication was done by Dr. Fennan and given the voucher specimen, which has been saved at the herbarium of the scientific institute # LHE.11. The bark of B. hispanica was removed, washed, dried in the shade, and then ground into fine powder. The plant extract was obtained by using maceration of 20 g of the plant powder in water for 24 h at ambient temperature. Afterward, the whole extract was undergone to filtration under reduced pressure [15,16].

2.2 Chemical characterization

Silvlation (TMS) is a derivatization technique that converts nonvolatile compounds into volatile derivatives through chemical derivatization [17]. GC-MS characterization of the plant extract was performed using the Claus 580 chromatography apparatus possessing a capillary column (5% phenyl and 95% methyplisyloxane; $30.0 \text{ m} \times 250 \mu\text{m}$) linked to a mass spectrometer (Polaris Q) (EI 70 eV). Helium was injected at 1 mL/min as a carrier gas. The injection volume was 1 µL, while the split was 1/75. Both injection and detection temperatures were 250 and 280°C. respectively. Furnace and regulating temperature was programmed as follows: first was set at 50 to 200°C with a rate of 11°C/min, and then from 200 to 240°C with a rate of 6°C/min. The identification of molecules was done by comparing the retention times obtained with those already used as database standards.

2.3 Animal material

Wistar albino rats weighing between 180 and 230 g were used as experimental animals for both acute and subchronic toxicity studies. Animals were acclimatized for 2 weeks under standard climatic conditions (22 ± 2°C and 12 hour light-dark cycle). Animals were fed with a chow pellet diet with free access to water. The Animal Ethics Review Committee of the Faculty of Sciences of Meknes, Morocco, reviewed and approved this study (#N° 201819K).

2.3.1 Acute toxicity

The acute toxicity study of B. hispanica was conducted according to the guidelines 423 [18]. Briefly, animals were divided into six groups each consisting of six rats (three females vs three males). Five different concentrations of the plant extract, 400, 800, 1,000, 1,200, and 1,400 mg/kg, were selected for administration into rats, and the control group received the same volume of vehicle (distilled water). Animals were studied for 14 days for recording clinical symptoms potentially induced by the plant extract. Clinical signs that occurred in mice including skin, mucous membranes, hair, eyes, respiratory, diarrhea, salivation, as well as lethargy were the most clinical symptoms noticed for treated rats under acute toxicity conditions [19]. The body weight of the treated animals was daily measured until the end of the experiment. Food and water intake alterations were also recorded for both female and male rats during the experimental period.

2.3.2 Subchronic toxicity

Subchronic toxicity of *B. hispanica* was assessed according to the guidelines 407 [20]. A group of 10 rats (five females vs five males) was treated with oral administration of 400 mg/kg/day of B. hispanica extract for 60 days, and the control group received the same volume of vehicle (distilled water) under subchronic toxicity conditions. Animals were controlled for clinical signs of toxicity during the whole experimental period, and the bodyweight was evaluated weekly.

2.3.2.1 Histopathological evaluation

At the end of the experiment, all animals were euthanized and subjected to cerebral dislocation. The kidney, liver, and lungs were excised to calculate the relative weight of organs.

The vital organs (kidney, lungs, and liver) were fixed in a 10% neutral formalin buffer for 24 h and then converted into paraffin cups using a rotary automaton. The tissue samples were subjected to the following process: dehydration into progressively increasing alcohol gradient, xylene cleaning, and immersion in wax. Afterward, the samples of organs were embedded in paraffin before thick sections being cut using a rotary microtome [21].

2.3.2.1.1 Hematoxylin and eosin staining

Histological sections were dewaxed in xylene and then rehydrated in decreasing concentrations of alcohol and then water. The slices were stained in hematoxylin for 5 min and washed with distilled water. Afterward, they were differentiated in 1% alcohol for 5 s and washed with tap water. The cups were colored in eosin for 10 min, washed with distilled water for 5 min, and then dehydrated in an increasing alcohol gradient. Finally, they were cleaned with xylene and covered with DPX support (a mixture of stilbene, tricresyl phosphate, and xylene) [22].

2.3.2.2 Analysis of serum biochemistry

The serum analysis was performed for rats treated under both acute and subacute toxicity conditions. At the end of

the experiment, all survived rats were euthanized and then subjected to cerebral dislocation for blood collection using heparin tubes. Afterward, the recovered blood was centrifuged at 2,500 tr/min for 15 min, and the obtained serum was used to perform the analysis of AST, ALT, urea, creatinine, and blood sugar using an automated analyzer according to the earlier protocol [19].

2.4 Statistical analysis

Data were expressed as mean \pm SD² using BioStat 2009 Professional 5.8.4 software. Student's t-test was used to perform analysis, and the values were statically considered at $(p \le 0.05)$

Ethical approval: This wok was approved by the Animal Ethical Committee of the Faculty of Sciences of Meknes, Morocco # Nº 201819 K.

3 Results

3.1 Chemical analysis of B. hispanica extract

The findings of chemical compounds detected in B. hispanica extract by GC-MS showed the presence of 13 compounds that majorly constituted of glucaric acid whose area percentage is 11.823 (Figure 1 and Table 1).

3.2 Acute toxicity

No symptoms occurred in rats treated with 400 mg/kg of B. hispanica extract under acute toxicity conditions. However, animals treated with 1,000, 1,200, and 1,400 mg/kg of the plant extract showed serious clinical symptoms in a dosedependent manner. Animals that were given oral administration of 1,400 mg/kg developed serious toxic symptoms like sedation and colored urine that could be due to inefficiency of renal elimination of the plant extract. Animals treated with the highest tested dose (1,400 mg/kg) also showed a dyspnea compared to the control group (Table 2). LD50 (lethal dose 50%) of B. hispanica extract was determined in 1016.16 mg/kg.

3.2.1 Food and water intake

Rats treated (males and females) with doses of 400 and 800 mg/kg of the plant extract under subacute toxicity

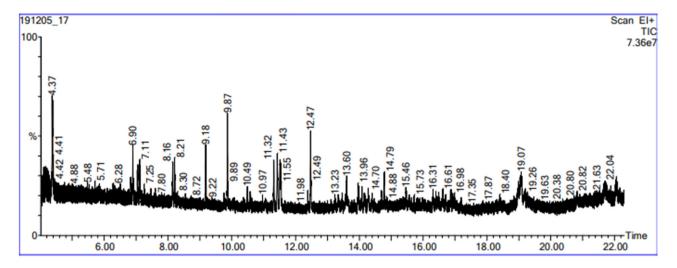


Figure 1: Chromatographic profile of B. hispanica bark extract.

conditions revealed no significant variations in water and food intake when compared to the untreated group (p > 0.05). However, a significant decrease in food and water intake was observed for animals treated with 1,000, 1,200, and 1,400 mg/kg when compared to the untreated group (p < 0.05; Figures 2 and 3).

day of treatment to reach a maximum at the end of the experiment when compared to the untreated rats (p < 0.05). Measuring bodyweight for female rats treated with the highest doses 1,200 and 1,400 mg/kg was interrupted due to the death of rats before the end of the experiment (Figures 4 and 5).

3.2.2 Bodyweight

During the whole period of dosing, acute toxicity findings showed a decrease in the bodyweight of animals (males and females) treated with a dose up to 1,400 mg/kg (Figures 4 and 5). Alteration of bodyweight of treated rats started progressively being significant from the eighth

3.2.3 Serum biochemistry analysis

Regarding serum biochemistry analysis for glucose, creatinine, AST, and ALT at the end of the experiment, the results obtained showed only significant increase in creatinine in blood collected from female rats treated orally with the highest dose (1,400 mg/kg) compared to the

Table 1: Phytochemical compounds identified in B. hispanica bark extract

| Retention time Molecular weight (g/mol) | | Compound name | Structural formula | |
|---|--------|---|---------------------------------|--|
| 4.371 | 126.24 | 2,4-Dimethyl-1-heptene | C ₉ H ₁₈ | |
| 6.90 | 184.36 | Decane, 2,5,6-trimethyl- | C ₁₃ H ₂₈ | |
| 7.71 | 286.41 | Oxalic acid, 2-ethylhexyl isohexyl ester | $C_{16}H_{30}O_4$ | |
| 8.16 | 196.37 | 6-Tridecene, 7-methyl- | $C_{14}H_{28}$ | |
| 9.18 | 198.39 | Tetradecane | C ₁₄ H ₃₀ | |
| 9.87 | 128 | Nonane | C ₉ H ₂₀ | |
| 11.32 | 200.36 | 11-Methyldodecanol | $C_{13}H_{28}O$ | |
| 11.55 | 168.32 | 1-Nonene, 4,6,8-trimethyl- | $C_{12}H_{24}$ | |
| 12.47 | 198.39 | Tetradecane, 7,8-dimethyl 7,8-dimethyltetradecane | C ₁₆ H ₃₄ | |
| 13.60 | 577.2 | 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane | $C_{18H_{52}O_{7}Si_{7}}$ | |
| 14.79 | 184.36 | Undecane, 3,7-dimethyl- | C ₁₃ H ₂₈ | |
| 19.07 | 441.4 | Folic acid | $C_{19}H_{19}N_7O_6$ | |
| 22.04 | 252.35 | Tertbutyloxyformamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]- | $C_{14}H_{24}N_2O_2$ | |

| Table 2: Clinical symptoms occurred in animals treated with B. hispanical | ca extracts under acute toxicity condi | tions |
|--|--|-------|
|--|--|-------|

| B. hispanica extract (mg/kg) | Number of rats treated | Mortality rate by sex | Total mortality rate | Clinical symptoms |
|------------------------------|---------------------------|-----------------------|-------------------------|---|
| 0 | 6 | 0/3 ♀ 0/3 ♂ | 0 | No symptoms |
| 400 | 6 | 0/3 ♀ 0/3 ♂ | 0 | No symptoms |
| 800 | 6 | 0/3 ♀ | 17 | Tachycardia, labored breathing, disorder, and loss of balance |
| | | 1/3 ♂ | | |
| 1,000 | 6 | 1/3 ♀ | 50 | Sedation, movement disorder, unconscious, fast heartbeat, labored breathing |
| | | 2/3 ♂ | | |
| 1,200 | 6 | 3/3 ♀ | 67 | Paralysis sedation |
| | | 1/3 ♂ | | |
| 1,400 | 6 | 3/3 ♀ 3/3♂ | 100 | Convulsion, total paralysis, coma, death |

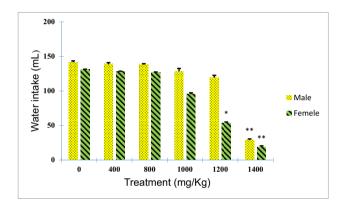


Figure 2: Effect of *B. hispanica* extracts on water intake (mL/100 g bodyweight) of animals treated with a dose up to 14,000 mg/kg.

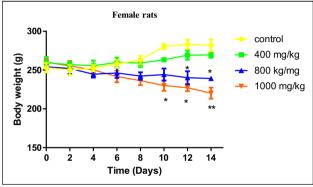


Figure 4: Effect of *B. hispanica* extracts on the bodyweight of female rats treated with a dose up to 14,000 mg/kg.

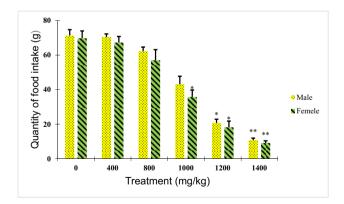


Figure 3: Effect of *B. hispanica* extracts on food intake (g/100 g bodyweight) of animals treated with a dose up to 14,000 mg/kg.

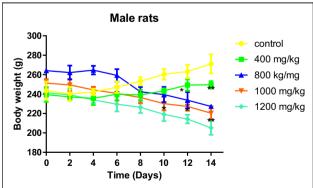


Figure 5: Effect of *B. hispanica* extracts on the bodyweight of male rats treated with a dose up to 14,000 mg/kg.

control group (*p < 0.05) (Table 3). Otherwise, there were no significant changes in blood collected from treated

animals (males and females) with a dose up to 1,400 mg/kg compared to the untreated group (p > 0.05; Tables 3 and 4).

Table 3: Effect of B. hispanica extract on biochemical parameters in female rats under acute toxicity conditions

| Parameters | Control | B. hispanica extract (mg/kg) | | | | |
|--------------------|------------------|------------------------------|-------------------|-----------------|-------------------|-------------------|
| | | 400 | 800 | 1,000 | 1,200 | 1,400 |
| Glucose (mg/dL) | 71.04 ± 1.72 | 74.25 ± 2.24 | 64.13 ± 2.12 | 73.54 ± 2.25 | 70.19 ± 5.64 | 68.34 ± 8.23 |
| Creatinine (mg/dL) | 0.82 ± 0.05 | 0.78 ± 0.05 | 0.85 ± 0.10 | 0.79 ± 0.12 | 0.44 ± 0.05 | $1.19 * \pm 0.25$ |
| AST (U/L) | 182.4 ± 4.55 | 177.33 ± 5.47 | 179.6 ± 17.46 | 188.25 ± 3.90 | 185.31 ± 4.85 | 189.01 ± 5.24 |
| ALT (U/L) | 49.4 ± 1.78 | 50.25 ± 6.31 | 54.27 ± 7.53 | 51.02 ± 2.43 | 55.75 ± 4.76 | 46.52 ± 5.17 |

^{*}Difference was significant.

3.3 Subchronic toxicity

Animals that received 400 mg/kg of *B. hispanica* extract orally under subchronic toxicity conditions showed serious clinical symptoms that adversely affected the animal bodyweight, which was progressively decreased throughout dosing when compared to the untreated group. There was a significant decrease in the bodyweight of the treated male rats from the first treatment days to reach a maximum at the end of the experiment compared to the untreated male rats (p < 0.05). There was a significant decrease in the bodyweight of the treated female rats from the 25th day of treatment more than ever before (p < 0.05; Figures 6 and 7). The findings obtained showed also a remarkable increase in the relative weight of organs (kidney, liver, stomach, and lungs) recovered from the treated animals when compared to the nontreated group ($p \le 0.05$; Figures 8 and 9).

3.3.1 Effect of B. hispanica extract on biochemical parameters

The findings of serum analysis showed a significant increase in the activity of plasma transaminase (ALT and AST) derived from the liver of both treated male and female rats when compared to the nontreated rats $(p^{**} < 0.01)$. However, the concentration of creatinine, urea, and blood sugar was not adversely affected when compared to the control rats ($p^* > 0.05$; Table 5).

3.3.2 Effect of B. hispanica extract on the internal organs

The results of the histological examination showed serious modifications in the tissues of liver, kidneys, and lungs in treated rats with B. hispanica extract (400 mg/kg/day) as follows: Regarding kidney parenchyma of animals treated with B. hispanica extract, serious histopathological injuries such as inflammation and renal and vascular congestion were detected (Figure 10d). The most histopathological modifications were found in the tissues of lungs, emphysema, and inflammation (Figure 10e). Moreover, the liver of the treated rats (Figure 10f) was also found to have degenerative alterations, sinusoidal and veins dilatation, vascular congestion, hemorrhagic foci, steatosis, and early periportal fibrosis.

4 Discussion

Herbal medicine has been used in the treatment of diseases many years ago. The Moroccan flora has largely contributed to fighting diseases that occurred in the local population and overall the neighboring countries [23]. Plants used for medication without scientific validity could negatively affect the health of the users [24]. Hence, it is important to pay attention to plants used in the prevention and treatment of diseases or used as food.

Table 4: Effect of B. hispanica extract on biochemical parameters in male rats under acute toxicity conditions

| Parameters | Control | B. hispanica extract (mg/kg) | | | | |
|--------------------|-------------------|------------------------------|-------------------|------------------|-------------------|------------------|
| | | 200 | 400 | 600 | 800 | 1,000 |
| Glucose (mg/dL) | 69.25 ± 3.34 | 62.98 ± 1.47 | 71.45 ± 4.32 | 81.15 ± 3.25 | 76.61 ± 1.46 | 73.11 ± 6.83 |
| Creatinine (mg/dL) | 1.14 ± 1.14 | 0.91 ± 0.25 | 1.01 ± 0.13 | 1.10 ± 0.04 | 1.09 ± 0.54 | 0.88 ± 0.02 |
| AST (U/L) | 110.75 ± 4.58 | 104.47 ± 3.25 | 112.73 ± 3.84 | 101.95 ± 3.45 | 114.07 ± 3.96 | 99.21 ± 4.09 |
| ALT (U/L) | 52.50 ± 3.08 | 49.10 ± 4.63 | 51.32 ± 8.05 | 55.02 ± 2.43 | 46.52 ± 6.17 | 55.75 ± 6.47 |

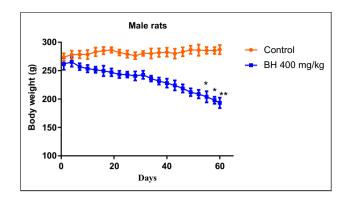


Figure 6: Effect of *B. hispanica* extracts on the bodyweight of the treated male rats under subchronic toxicity conditions.

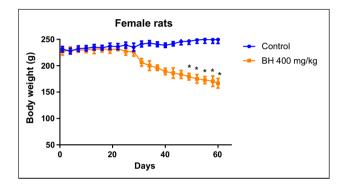


Figure 7: Effect of *B. hispanica* extracts on the bodyweight of the treated female rats under subchronic toxicity conditions.

This study was conducted to study the acute and subchronic toxicity of B. hispanica bark extract used in Morocco pharmacopeia. Under subchronic toxicity conditions, animals treated with B. hispanica extract with a dose up to 1,400 mg/kg showed serious clinical symptoms that could be attributed to toxic properties induced by the extract administration [19]. The dose of *B. hispanica* bark causing mortality in 50 percent of animals (LD50) was estimated at 1016.16 \pm 224.57 mg/kg, and therefore, the plant extract is considered slightly toxic since 1016.16 ± 224.57 mg/kg falls within the scope 500-5,000 mg/kg [25]. Under subchronic toxicity conditions, it came to our attention that male rats were more sensible to plant extract toxicity compared to female rats. This observational remark was consistent with the previously reported findings, which showed that males have a longer gastric retention time that delays food absorption and, therefore, increases adverse drug reactions [26].

Changes in food and water intake were frequently used as indicators for the assessment of the general health status of animal experimentation [27]. Under subacute toxicity conditions, a decrease in water and food intake of animals (males and females) treated with

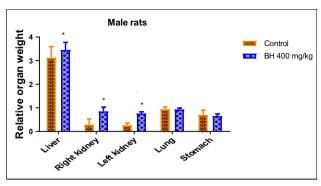


Figure 8: Effect of *B. hispanica extract* on the relative weight of organs (kidney, liver, stomach, and lungs) of the treated male rats under subchronic toxicity conditions.

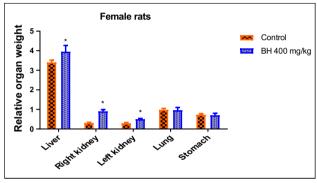


Figure 9: Effect of *B. hispanica* extract on the relative weight of organs (kidney, liver, stomach, and lungs) of the treated female rats under subchronic toxicity conditions.

an increasing dose up to 1,400 mg/kg was observed during the whole period of dosing, and therefore, we suggest that animals were negatively affected by the plant extract. A decrease in both food and water intake was observed for treated animals due to the deregulation of appetite under the plant extract effect.

Under acute and subchronic toxicity conditions, a remarkable decrease in the bodyweight of animals (males and females) treated with an increasing plant extract dose up to 1,400 mg/kg was observed. This result can be naturally explained by decreasing the food and water intake in animals treated with the plant extract. This sign of toxicity can also result from the deregulation of appetite. These findings were closely in accordance with the previously reported literature, which revealed a loss of animal weight due to drug-induced toxicity [28].

The analysis of biochemical parameters for treated animals provided data on plant extract-induced toxicity under acute and subchronic toxicity conditions. ALT and AST are enzymes found normally in the liver; however, when hepatic cells got damaged under diseases or toxic

Table 5: Effect of *B. hispanica* extract on biochemical parameters

| Biochemical parameters | Control | Treated rats (400 mg/kg) | |
|------------------------|--------------------------------|-----------------------------|--|
| Females | | | |
| AST (U/L) | 103.5 ± 2.2 | 152.20 ± 5.4* | |
| ALT (U/L) | 43.60 ± 1.1 | 93.75 ± 3.3* | |
| Urea (mg/dL) | 63.96 ± 2.1 | 67.96 ± 3.21 | |
| Blood sugar (g/L) | 1.18 ± 0.1 | 0.91 ± 0.1 | |
| Creatinine (µmol/L) | 0.60 ± 0.12 | 0.83 ± 1.2 | |
| Males | | | |
| AST (U/L) | 131.25 ± 8.7 | 212.75 ± 5.4* | |
| ALT (U/L) | 76.75 ± 4.4 | 123.75 ± 6.12** | |
| Urea (mg/dL) | 58.06 ± 4.16 | 62.14 ± 6.13 | |
| Blood sugar (g/L) | 1.02 ± 0.03 | 0.97 ± 0.07 | |
| Creatinine (µmol/L) | $\textbf{1.02}\pm\textbf{0.8}$ | 1.36 ± 0.7 | |

^{*}Difference was highly significant.

agents, these enzymes are discharged into surrounding extracellular space [29]. Under acute toxicity conditions, significant increase in only creatinine was observed in the blood collected from female rats treated orally with the highest tested dose (1,400 mg/kg), and therefore, we confirm that the tested extract has little toxicity vs kidneys when ingested in a single dose up to 1,400 mg/Kg. Under subchronic toxicity conditions, the plasmatic activities of ALT and AST dosed in recovered plasma of treated rats with extract were higher than those of the nontreated group $(p \le 0.05)$, and consequently, the extract studied revealed to be drug-induced liver injury. The potential extract-induced kidney damage was also assessed by measuring the concentration of plasma urea and plasma creatinine, and the results revealed no significant variation when compared to the untreated group (p > 05). The results obtained were in agreement with earlier results, which showed the elevation of plasma creatinine and plasma urea concentration under chemical-induced kidney injury [19].

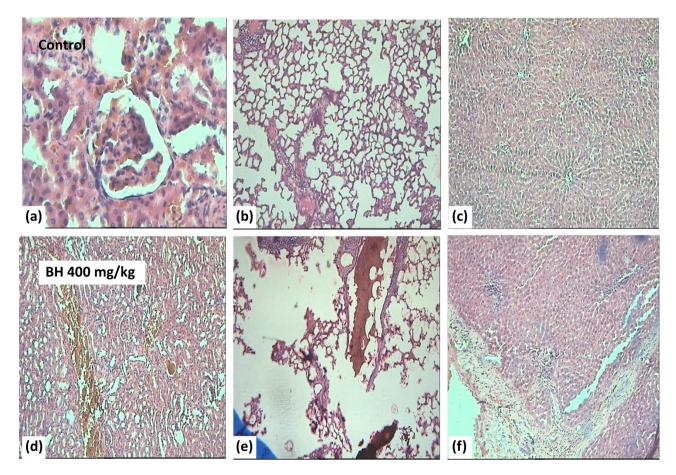


Figure 10: Effect of *B. hispanica* extracts on the internal organs (kidney, lungs, and liver) of animals treated with 400 mg/kg/day. (a) Sections of kidney parenchyma of untreated rats; (d) sections of kidney parenchyma of treated rats; (b) sections of lung parenchyma of untreated rats; (e) sections of lung parenchyma of treated rats; (f) sections of liver parenchyma of treated rats.

^{**}Difference was extremely significant.

Risk assessment of *B. hispanica* bark extract in rats under chronic toxicity conditions was also investigated by analyzing the internal organ tissues such as liver, kidney, and lungs. Moderate clinical injuries were recorded in the kidney and lungs; meanwhile, the histopathological changes were more pronounced in the liver. These findings were in confirmation with those obtained from serum analyses since no changes were reported in urea and creatinine; however, serious elevation was detected in transaminase activities controlled by the liver. The major histopathological changes revealed in the liver of rats treated with 400 mg/kg/day were degenerative alteration, sinusoidal and veins dilatation, vascular congestion, hemorrhagic foci, and steatosis. These findings were consistent with those reported in the earlier data, which showed similar histological modifications that occurred in the internal organs of treated rats with Berberis vulgaris [30]. Kidneys of animals treated with B. hispanica extract showed inflammation and renal and vascular congestion, and therefore, these symptoms could indicate extract-induced nephrotoxicity as reported in the literature [19]. Toxicities occoured under both subacute and subchronic toxicity conditions in rats is mainly attributed to compounds detected in B. hispanica bark extract. These compounds can react individually or in synergy without excluding potential potentiation effects.

The genus *Berberis* belongs to the family Berberidaceae including more than 500 species [11]. The principal isolated compounds from *Berberis* were reported to be phenols, tannins, triterpenes, sterols, and alkaloids [29–32]. Alkaloids contained in *Berberis* are revealed to be toxic in mice under subchronic toxicity by inducing the increase of plasma activity of ALT and AST, which are liberated by damaged cells of the liver [32,33]. Hence, closer attention should be paid to plants used in traditional medicines for therapeutic purposes.

5 Conclusion

This study can provide more data on the phytochemical composition and *in vivo* toxicity of *B. hispanica*. The outcome of this study showed that *B. hispanica* extract revealed to be toxic in rats that received an oral dose higher than 400 mg/kg under subchronic toxicity conditions. The studied extract showed also serious toxicities in rats that received 400 mg/kg under subchronic toxicity conditions. Consequently, particular attention should be paid to the scientific validity of plants used in traditional medicines.

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