Abstract: To investigate the phytochemical composition, acute and sub-acute toxicity of the aqueous extract of *B. dioica* roots. The phytochemical analysis was performed using gas chromatography-mass spectrometry (GC-MS). The acute toxicity of the aqueous extract of *B. dioica* roots was assessed in mice with single doses ranging from 250 to 1000 mg/kg for 14 days. The sub-acute toxicity was carried out with repeated doses ranging from 64.5 to 250 mg/kg for 28 days. Histopathological changes and markers of renal and liver function were investigated. The results of GC-MS analysis showed the presence of interesting phytoconstituents. The clinical symptoms and mortalities that occurred in treated mice were more remarkable due to the increasing sample concentration of the studied extract. However, no mortalities, or histopathological, or biochemical disturbances were observed even at the maximal dose administered (250 mg/kg). The outcome of the present work suggests that the treatment of animals with single doses of *B. dioica* roots extract higher than 250 mg/kg produces significant toxicities, however, treatment with repeated doses up to 250 mg/kg for 28 days seems to be safe for animals.

Keywords: acute toxicity; sub-acute toxicity; histopathology; biochemistry; GC-MS.

1 Introduction

For many years herbs and plants have been used to maintain good health. They have been found to have medicinal and therapeutic importance in the prevention and treatment of diseases [1]. Evidence of the use of herbs as medicine by early people can be traced back to 60,000 years ago [2]. Plants are naturally producing secondary metabolites when they face potential threats from the surrounding environment [3]. Synthetic drugs are often coupled with a number of secondary effects. Medicines based on natural herbs could generate fewer or no secondary effects and as a result are still attractive in human communities [4]. Nowadays, about 80% of the population throughout the world rely on traditional herbal medicine in order to meet their health care needs [5]. The use of medicinal plants without paying attention to their toxicities may have presented an enormous aggravation to humans. It is widely probable that when searching for food, early people often ingested poisonous herbs which could induce symptoms of toxicity such as diarrhea, vomiting, coma, or other toxic reactions which over time may lead to death. To counteract this early humans were able to innovate and develop knowledge about consumable materials [6]. The safety of medicinal herbs has become a major source of challenge in using natural preparation [7]. For this reason, it is very important to validate the safety of herbal medicines before their use and scientific data collected from the toxicological screening of medicinal plants could be used to build confidence for human uses [8].

Many people believe that traditional medicines are safe due to their natural origin. In developing countries, medicinal plants are often randomly used in the treatment of diseases without scientific proof. In spite of the large use of herbal medicine for health care in developing countries, no significant scientific data is available regarding their potential toxicities [9]. Some medicinal plants could be toxic and thus may induce negative impacts on human
health. In order to ensure the safety control of plants or their derivatives, systematic studies starting with the toxicological profile are required to conjecture risks of toxicity, and thus providing scientific data for selecting doses that could be safe for humans [10].

*Bryonia dioica* (*B. dioica*), is a wild plant with tuberous roots. Belonging to the genus Bryonia some species are toxic [9]. This plant is frequently used in old medicine to cure diseases such as inflammatory conditions, joint pain, pleurisy, intestinal ulcers, fevers, bronchitis, asthma, arthritis, hypertension, and diabetes [11]. In Morocco, *B. dioica* has been used in the treatment of stomach ache, rheumatic pains, liver failure, colds, ulcer, dysentery purgative[12], and cancer [13].

To the best of our knowledge, no previous study has been carried out on the toxicological profile of aqueous extract of *B. dioica* roots. Therefore, the current work was conducted in vivo for screening a potential risk of *B. dioica* roots used in north-African alternative medicine.

2 Materials and Methods

2.1 Plant sample collection and identification

Plant material was collected in December 2016 from the surrounding region of Casablanca. The plant was identified and has been deposited under N°101547 in the Scientific Institute of University Mohammed V–Rabat–Morocco. The roots were initially cleaned, washed with water, left to dry at room temperature then ground into small pieces. 50 g of dried powder was boiled in water for 20 minutes at 100°C according to the method described in earlier literature [14]. The mixture was filtered and concentrated in a rotary vacuum evaporator under reduced pressure to yield 5 g of crude extract.

2.2 Identification of bioactive constituents by GC-MS

GC-MS analysis of the extract of *B. dioica* roots was performed using a Claus 580 Gas chromatograph conducted under to the following acquisition parameters: Oven: Initial temp 50°C for 2 minutes, ramp 11°C/min to 200°C, hold 0 minutes, ramp 6°C/min to 240°C, hold 1 minute, Inj Bauto=0°C,Volume=0 µL, Split=10:1, Carrier Gas=He, Solvent Delay=4.00 min, Transfer Temp=280°C, Source Temp=250°C, Scan: 40 to 450 Da, Column 30.0m x 250 µm

2.3 Animal subjects

Swiss albino mice (males) weighing approximately 25 g procured from the animal colony of Pasteur Institute-Morocco were used to perform the current work. The mice were housed under standard environmental conditions; light/dark cycles (12/12 hours), temperature (24 ± 2°C), ventilated place. The mice had fed with standard laboratory pellets and had free access to water. Each cage includes 6 mice with a bedding of husk. The animals were segregated according to their body weight.

2.4 Acute oral toxicity studies

All animals were retained as per the approval of the guide for the care and use laboratory animals approved by the institutional animal’s committee at University Hassan II under (OECD) Guidelines No.425 (OECD, 2008) [15]. After fasting them overnight, the animals were divided into 4 groups (one control and three treated groups). Each group includes 6 mice (males). Three different doses of *B. dioica* roots extract were selected to be administered orally in single doses for 14 days (250, 500 and 1000 mg/kg body weight). The control group were administered with distilled water. The animals were observed continuously for clinical symptoms during the whole period of the experiment [16].

2.5 Subacute toxicity study

The 24 male mice were segregated into 4 groups (6 per group) and were used in the subacute toxicity studies of aqueous extract of *B. dioica* roots. Three different doses of the plant extract were administered daily for 28 days to three groups and the fourth, the control group, received distilled water. The groups were designed as follows:

| Group | Treatment |
|-------|-----------|
| A     | *B. dioica* roots (62.5 mg/kg/day) |
| B     | *B. dioica* roots (125 mg/kg/day) |
| C     | *B. dioica* roots (250 mg/kg/day) |

All animals were handled according to the approval of the guide for the care and use laboratory animals approved by the institutional animal’s committee at University Hassan II with respect to the Organization for Economic Cooperation Development (OECD) Guideline No.287 [17]. The animals were observed for signs of toxicity and mortality and thus weighed each week during the whole
Phytochemistry and toxicological assessment of *Bryonia dioica* roots used in north-African culture. The experiment period [18]. The weight change was calculated according to the following formula:

\[
\text{Percentage Gain} = \left( \frac{\text{Weight Gain}}{\text{Original Weight}} \right) \times 100\%
\]

### 2.6 Serum biochemical analyses

At the start of the treatment period all mice were fasted overnight and sacrificed for blood collection. Blood samples were collected from the orbital sinus. The biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), Urea and Creatinine were measured enzymatically using an automated analyzer.

### 2.7 Histopathological profile

At the end of the treatment period the treated mice were sacrificed for organ collection. The vital organs, such as liver and kidney were extracted and carefully fixed in a 10% formalin solution then proceeded for histopathology. Serial sections were made and stained with Haematoxylin-Eosin (H&E), then subjected to gradient dehydration, hyalinizing and sealing film. The pathological lecture of tissue was effectuated by a pathologist using a light microscope [19].

### 2.8 Statistical analysis

Quantitative data of difference in biochemical parameters, body weights for both treated and control mice were analyzed according to mean values ± SD (standard deviation). The significance of the difference between the means was determined by one-way ANOVA using GraphPad Prism 7 software. The means were compared using the Tukey test. Statistically, the difference was considered significant at P<0.05.

### 3 Results and Discussion

#### 3.1 GC-MS analysis

The GC-MS analysis of *B. Dioica* extract revealed the presence of six compounds; 1, 2,3-triméthylbenzène, Coumaran, 2-Formyl-9-[á dRibofuranosyl]hypoxanthine, Maaliol, 3-méthylglucose and Phthalic acid, di(2-propylpentyl) ester (Figure 1; Table 2).

The gas chromatography-mass spectrometry analysis of *B. dioica* roots extract revealed the presence of six compounds; 1, 2,3-triméthylbenzène, Coumaran, 2-Formyl-9-[á dRibofuranosyl] hypoxanthine, Maaliol, 3-méthylglucose, Phthalic acid, di(2-propylpentyl) ester. It seems that the pharmacological properties of *B. dioica* roots reported in the current research study were frequently attributed to the detected bioactive molecules by GC-MS. It was reported that the bioactive compounds

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**Figure 1**: GC-MS chromatogram of *B.dioica* extract
revealed in the studied extract could dominate the acute toxicity results due to the action of a single molecule or a synergistic effect. Particularly, the detected compound in the plant extract like Phthalic acid, di(2-propylpentyl) ester as reported in an earlier report exhibiting a toxic effect on brine shrimp nauplii *Artemia salina* [20].

### 3.2 Acute toxicity studies

During the whole period of the acute toxicity study there were no mortalities nor signs of toxicity in treated mice with doses up to 250 mg/kg. However, the treatment with a dose of 500 mg/kg induced several symptoms of toxicity for example hypoactivity and occasional convulsions compared to the control group. The second tested dose (500 mg/kg) was responsible for 50% of death in mice within 24 hours. At the highest dose administered (1000 mg/kg), the mortality rate increased to 83% accompanied by remarkable signs of toxicity, such as abnormal locomotion, hypoactivity, occasional convulsion, and reversal reflection. The results of the acute toxicity studies revealed that the maximum tolerated dose by mice was 250 mg/kg body weight. The LD₅₀ (the amount of plant extract required to kill 50% of the test population) of the plant extract was determined at 500 mg/kg by oral administration. According to Globally Harmonized System of Classification and Labeling of Chemicals (GHS) approved by the OECD (EU classification), the aqueous extract of *B. dioica* roots may be classified as a class 4 and thus considered as a low toxic substance [21]. The clinical signs of toxicity occurred in mice under conditions of acute toxicity, such as hypoactivity, lack of appetite, occasional convulsion and reversal reflection which may be attributed to properties of the plant extract [22]. Many earlier data reported toxic effects of *Bryonia* spp, which could be induced by triterpenic, cucurbitacins, glycosides or their derivatives present in various parties of the plant. Cucurbitacins are famous for their cytotoxic effects and have hence been suggested to be used as potential anticancer agents [23]. Bryodiofine a toxic protein particularly current in the fruits of *B. dioica* could also be responsible for intoxication occurred in children. Many clinical symptoms occurred in humans due to the ingestion of *B. dioica* fruits like convulsions, vomiting, nausea, abdominal pain and diarrhea, sweating, pallor, respiratory and cardiac disorders as reported in earlier reports [28].

### 3.3 Sub-acute toxicities

During the whole period of treatment no clinical symptoms of toxicity nor animal death were observed due to the administration of the tested doses under the subacute toxicity conditions. Slight changes in general behavior like running about 2 to 3 minutes after the gavage were observed compared to the control group which may be related to animal handling.

#### 3.3.1 Effect of aqueous extract on the mice’s weight during the treatment period.

According to data presented in Figure 2 during the whole experiment period no significant changes occurred in the weight of treated groups with a dose up to the maximum of 250 mg/kg compared to the control group (p>0.05).

The weight alteration is considered an important indicator for the assessment of early signs of toxicity induced by drugs and chemicals [15]. As shown in Figure 2, the mice treated during the experiment period gained weight compared to the control group (p > 0.05). It may be ended that the oral administration of aqueous extract

### Table 2: Phytocomponents identified in *B.dioica* roots extract by GC-MS analysis.

| S.No | Retention time (min) | Compound name | Molecular formula | CAS       | Peak area % |
|------|----------------------|---------------|-------------------|-----------|-------------|
| 1    | 6,243                | 1,2,3-triméthylbenzène | C₉H₁₂      | 526-73-8  | 1,877       |
| 2    | 9,875                | Coumaran      | C₈H₈O      | 496-16-2  | 2,124       |
| 3    | 12,913               | 2-Formyl-9-[α-d-ribofuranosyl] | C₅H₅N₄O₆ | 125425-35-6 | 2,614       |
| 4    | 14,097               | hypoxanthine  | C₇H₇O      | 527-90-2  | 1,712       |
| 5    | 15,669               | Maaliol       | C₇H₁₄O₆     | 146-72-5  | 29,412      |
| 6    | 19,438               | 3-méthylglucose | C₇H₁₄O₆    | 70910-37-1 | 1,198       |
of *B. dioica* with doses up to the maximum of 250 mg/kg for 28 days did not affect negatively the normal growth of oral treated mice.

### 3.3.2 Effect of aqueous extract on biochemical parameters

The results of the clinical biochemistry parameters assessed in this work, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), Urea and Creatinine are summarised in Figure 3. It can be seen that there were no significant changes observed in groups treated with doses up to the maximum of 250 mg/kg compared to the control group (p>0.05).

The transaminases (ALT, AST) are considered to be critical markers of liver function, and a significant increase of their plasmatic levels is highly related to hepatic cytolysis [17]. The hepatic function, assessed in this work by measuring the serum levels of ALT and AST in the blood of treated groups with doses up to the maximum 250 mg/kg, showed that the aqueous extract of *B. dioica* roots did not negatively affect the liver function compared to the controls (p>0.05). These results were comparable to those reported in previous data [11], in which it was reported that the organic extract of *B. dioica* offered a hepatoprotective action against hepatotoxins agent (CCl4). The current results of biochemical parameters were also in agreement with those reported in histopathological examination investigated in the present work, which showed no injuries occurred in liver tissue. The kidneys are among the most vital organs which susceptible to toxic compounds because of the high volume of blood that flows through them. They filter many types of toxins, which could be accumulated in their tubules [26]. Urea and creatinine as critical biomarkers were often measured for assessing the renal function [26]. In our study, no significant difference observed in the creatinine nor urea concentration of treated groups with doses up to 250 mg/kg compared to the control group (P> 0.05). The increased plasmatic level of lactate dehydrogenase (LDH) is till frequently related to cell damage [28]. Considering the LDH activity assessed in the current study, no significant changes were noted in the serum of treated groups with the tested doses compared to the control group, (P> 0.05). Hence, all the tested doses in the current study seem to be safe in mice.

### 3.3.3 Histopathological profile

#### 3.3.3.1 Kidneys

The kidneys play a crucial role in maintaining stable physiological conditions as they perform several important functions for example maintaining the necessary amount of water required for body functions and eliminating the waste. The kidneys manage and eliminate metabolic waste, maintain minerals and other substances in the blood and body fluids at an adequate level. The function of kidneys is not limited to these biological properties they also produce hormones, regulate blood pressure and produce red blood cells [28]. The kidneys have a high sensitivity to toxins due to the high volume of blood which flows through them and their normal function can be assessed by histopathological or biochemical examination of some parameters contained in the biological fluids like urea and creatinine. The results of the microscopic examination of
Figure 3: The effect of the oral administration of aqueous extract of *B. dioica* roots on AST, ALT, creatinine, urea, and LDH of treated mice, results as the mean ± SD (standard deviation).
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renal tissue stained with hematoxylin and eosin showed no histopathological changes that occurred in the kidneys of the mice treated with doses up to a maximum of 250 mg/kg compared to the control group as shown in Figures 4 and 5. These findings were also in agreement with those reported in the biochemical evaluation, which showed the absence of biochemical alterations in urea and creatinine in the blood of the treated mice with increasing sample concentration. The increased plasmatic concentration of these parameters is often accompanied by nephrotoxicity, hence, the aqueous extract of *B. dioica* roots seems to be safe for kidneys of oral treated mice with doses up to the maximum of 250 mg/kg under the subacute toxicity conditions of.

3.3.3.2 Liver

The liver is one of the most vital organs playing a key role in energy exchange, synthesis of proteins, metabolism regulation, elimination of waste produced in the body, storage and distribution of nutrients from the intestine. The liver continuously receives toxic substances and converts them to nontoxic products under the liver detoxification reaction. Both histological and biochemical examination are the classic methods used for assessing the liver function. In terms of biochemical evaluation, the liver function is assessed by dosing some serum activities of their products like AST and ALT which are found normally inside hepatic cells. Liver alteration is often compensated by an increase of the plasmatic activities of AST and ALT over the biological constant [29]. The findings of the microscopic examination of liver tissue stained with hematoxylin and eosin showed

Figure 4: Histologic section of kidney tissue of the control mice. (Section stained with H&E, x 40) A, B, and C are sections of the kidney tissue of three mice taken from the control group.

Figure 5: The effect of the oral administration of aqueous extract of *B. dioica* roots on the kidney tissue of treated groups with doses up to the maximum of 250 mg/kg (Section stained with H&E, x 40) section of renal tissue of treated group with 62.5 mg/kg, (B) section of renal tissue of treated group with 125 mg/kg, (C) section of renal tissue of treated group with 250 mg/kg.
no remarkable histopathological injuries occurred in the liver of oral treated groups with doses up to the maximum of 250 mg/kg compared to the control group as shown in Figures 6 and 7. These results were in agreement with those reported in serum analyses of biochemical parameters such as aspartate aminotransferase and alanine aminotransferase which were unaltered during the whole period of oral administration of the aqueous extract. These results were also used for performing a comparison with those reported in previous studies [10]. It showed that the ethanolic extract of *B. dioica* roots offered a hepatoprotective activity in mice treated with (CCl4). Considering these results, the aqueous extract of *B. dioica* roots seems to be safe for the liver of oral treated mice with a dose no higher than 250 mg/kg under the subacute toxicity conditions of.

The present results could indicate that the aqueous extract of *B. dioica* roots at the studied doses did not negatively affect the renal or liver function. These findings were also supported by those registered in histology examination, which showed no histopathological changes that occurred in oral treated groups with doses up to the maximum of 250 mg/kg. Based on the current results of subacute toxicity, all doses tested seem to be safe for mice.

### 4 Conclusion

Acute oral administration of aqueous extract of *B. dioica* roots with a dose higher than 250 mg/kg was shown to be toxic for mice under the acute toxicity conditions of. The aqueous extract of *B. dioica* seems to be safe for animals with doses up to 250 mg/kg under the subacute toxicity conditions of. The outcome of the present work demonstrates the importance of understanding the scientific validity and toxicity of plants used in traditional medicine.
Conflict of interest: The authors declare that there are no conflicts of interest.

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