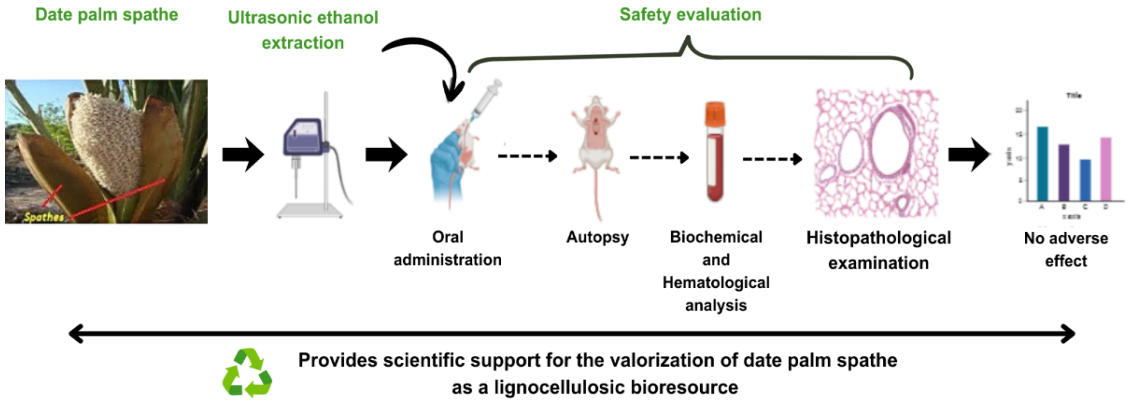
# Safety Assessment of Polyphenolic Extracts from Date Palm Spathe as a Valorizable Bioresource

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## GRAPHICAL ABSTRACT



# Safety Assessment of Polyphenolic Extracts from Date Palm Spathe as a Valorizable Bioresource

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The spathe of the date palm *Phoenix dactylifera* (L.) is a lignocellulosic agricultural by-product that can be exploited as a source of biomass for high value products. However, limited information is available regarding the toxicological safety of its extracts. This study aimed to evaluate the *in vivo* toxicity of date palm spathe extracts through acute and subacute oral tests in mice to support their potential valorization. In the acute test, female mice were treated with single oral doses of 300 and 2000 mg/kg for 14 days. For the subacute test, daily doses of 50, 300, and 1000 mg/kg were administered to both sexes for 28 days. In both studies, clinical symptoms, mortality, body weight variations, organ weights, biochemical and hematological criteria, and histopathological analyses were examined. No lethality or significant clinical signs were observed at any of the tested doses. Hematological and biochemical parameters showed no significant changes between groups. Histopathological examination of the organs showed similar results in both the acute and subacute phases, represented by mild, non-specific changes compatible with background lesions in mice. These findings indicate a favorable toxicological safety profile, supporting the potential valorization of date palm spathe extracts.

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*Keywords: Phoenix dactylifera (L.); Date palm spathe; Lignocellulosic waste; Ethanolic extract; In vivo safety*

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## INTRODUCTION

The date palm, *Phoenix dactylifera* (L.), which belongs to the Arecaceae family, is an iconic fruit-bearing plant species of arid and semi-arid regions. It has been cultivated for centuries and called nakhla by the Arabs. Beyond its agro-economic importance, it plays an essential role in the cultural, nutritional, traditional and medicinal perspectives of many communities in the Middle East, Arabian Gulf, and North Africa (Vyawahare *et al*. 2009). It is a robust and versatile tree that withstands hot climatic conditions, has numerous

cultivars and varieties with unique characteristics, and constitutes a strategic resource for food security and sustainable development of desert regions owing to the significant production of dates (Al-Karmadi and Okoh 2024).

The annual production of dates generates a considerable quantity of agricultural by-products, nearly 2 million tons worldwide. These byproducts include seeds, dried fruits, palms, leaves, and fibers, which are often discarded as waste despite their diverse chemical compositions, presenting strong potential for valorization. Date palm residues constitute a renewable and abundant natural source with highly variable properties, although transformation processes remain to be developed for several applications (Agoudjil *et al*. 2011; Faiad *et al*. 2022). Numerous studies have highlighted their traditional uses, nutritional values, and medicinal and biological properties, suggesting their importance as functional ingredients that can be exploited in various sectors (Mahomoodally *et al*. 2023).

Among these wastes, spathes are one of the major lignocellulosic residues that accumulate in large quantities each year during the pollination of date palms. The spathe is a rigid and fibrous envelope that protects the inflorescence during its growth period and opens when the flowers reach maturity. It is green to brown in color and has a specific odor, particularly when fresh. It is usually removed during manual pollination of date palms (Bouhoreira *et al*. 2016). Spathes play an important role in protecting the reproductive organs of the date palm, which is a dioecious species in which male and female flowers develop on two distinct trees (Atghaei *et al*. 2020).

The chemical composition of the spathe of *Phoenix dactylifera* (L.) revealed that this residue is an excellent source of volatile compounds (Jahromi *et al*. 2014), secondary metabolites, particularly polyphenols and flavonoids (Asadi *et al*. 2025), as well as proteins, sugars, fats, and amino acids (Al-Okbi 2022). This composition endows the spathe with multiple interesting biological properties, including antioxidant (Farboodniay *et al*. 2016), antimicrobial (Al-Zoreky and Al-Taher 2015, 2019), anti-inflammatory, and analgesic effects (Peyghambari *et al*. 2015). These properties open the way for the valorization of the spathe as a natural source of high-value biomolecules intended for the development of nutraceutical, pharmaceutical, agri-food, and cosmetic products.

Despite the traditional use and pharmacological effects of spathe, scientific data regarding the toxicological safety of its use remain extremely limited in the literature. The use of any substance, including plant extracts, does not automatically ensure safety, as toxic effects may occur depending on the dose, duration of exposure, or type of extract used. At the same time, the toxicity of plant extracts is not necessarily undesirable and may be viewed differently depending on the intended application. While the absence of toxic effects is crucial for applications related to human or animal health, controlled toxicity may be desirable in other areas, such as agriculture. Therefore, a comprehensive toxicological profile must take into account both safety for human exposure and potential bioactivity for other value-added applications. Establishing the safety profile of *Phoenix dactylifera* spathe is crucial to define its therapeutic window. This window represents the dosage range between the minimum effective concentration and the threshold of adverse effects, ensuring that beneficial properties can be exploited without compromising organism safety (Nair and Jacob 2016). As a first step toward this comprehensive profile, the present study aimed to rigorously and thoroughly evaluate, for the first time to our knowledge, the safety of spathe extracts to fill this gap in scientific studies. Consequently, toxicity evaluation was conducted through acute and subacute toxicity analyses based on hematological, biochemical, and histological tests. This approach is an essential step for understanding the

safety profile of this lignocellulosic residue and providing crucial data to ensure safe and rational valorization.

## EXPERIMENTAL

### Plant Material and Polyphenol Extraction

Samples of the spathes of the date palm *Phoenix dactylifera* (L.) were collected from trees grown under natural field conditions, not in a controlled herbarium setting, at the Technical Institute for the Development of Saharan Agronomy (ITDAS) in Biskra, Algeria (latitude 34°42′ N, longitude 5°47′ E), This region has a typical Saharan arid climate, with very low annual rainfall, hot summers, and mild winters. The soil is sandy-loam, which is characteristic of Saharan agricultural zones. Male spathes were harvested during the flowering period in March 2022, while female spathes from *Mech Degla* cultivars were collected in October 2022, corresponding to their optimal maturity phase. Immediately after harvest, the plant material was carefully freed of impurities, dried away from light at ambient temperature, and ground into a fine powder using an electric mill. This powder was stored in airtight bottles, protected from light and humidity, until the extraction step.

Polyphenol extraction was performed at the Scientific and Technical Research Center for Arid Regions (CRSTRA) using the ultrasound-assisted extraction method (Sonics and Materials Inc., Newtown, USA) according to the protocols described by (Bourgou *et al*. 2016; Dzah *et al*. 2020), with slight modifications. In brief, 10 g of spathe powder (male and female) was mixed with 100 mL of aqueous ethanol (70%, v/v) and sonicated in an ultrasonic bath (20 kHz, 200 W) for half an hour at a controlled temperature (30 ± 5 °C) using a cooled water bath to avoid thermal degradation of the polyphenols. The extracts were then filtered using a vacuum filtration pump, concentrated using a rotary evaporator under low pressure at 40 °C, and stored in amber glass bottles at 4 °C until use. Several extractions were performed to obtain the required quantity of extract for the experiment.

### Quantitative Analysis of Phenolic Compounds

*Total phenolic content*

The total phenolic content (TPC) of the male spathes (S-M) and female spathes (*Mech Degla* variety S-MD) extracts was determined using the Folin-Ciocalteu colorimetric method (Singleton and Roosi 1965) and according to the microplate assay described by Müller *et al*. (2010). The protocol consisted of mixing 20 µL of extract with 100 µL of diluted (1:10) Folin-Ciocalteu reagent. After a stand time of a few minutes to allow the initial reaction to occur, 75 µL of 7.5% sodium carbonate ($Na_2CO_3$) was added. The mixture was then incubated in the dark at room temperature for 2 h. Absorbance was measured using a microplate reader at 765 nm. The phenolic content was calculated using a gallic acid calibration curve, and the results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

*Total flavonoids content*

The total flavonoid content (TFC) in the extracts was determined based on the formation of a stable complex between $Al^{+3}$ and the hydroxyl groups of the flavonoids. Following a standard protocol, 50 µL of the extract was mixed with 130 µL of methanol,

followed by 10 µL of potassium acetate ($CH_3COOK$). Then, 10 µL of aluminum nitrate was added to the mixture. After an incubation period of approximately 40 min in the dark at room temperature, absorbance was measured at 415 nm (Topçu *et al*. 2007). The flavonoid content was determined using a quercetin calibration curve, and the results were expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

## Experimental Animals

Acute and subacute toxicity tests were conducted at the Laboratory of Veterinary Pathological Anatomy and Cytology (LACPV) of the Pasteur Institute of Algeria. Male and female NMRI mice (*Mus musculus*), aged 6 to 7 weeks with an average weight of 20 to 25 g, were used in this study. All experimental animals were obtained from the animal facility at the Pasteur Institute and housed in plastic cages under controlled temperature (25±5 °C), relative humidity (50% to 60%), and a 12-h light/dark cycle, with free access to standard pellet diet and water. A 7-day adaptation period was observed before the experimentation. The experiment was conducted following Organization for Economic Cooperation and Development (OECD) recommendations, as well as institutional protocols and guidelines for the care and use of laboratory animals (National Research Council 2011). Throughout the study period, all animals were treated humanely in accordance with the international guidelines for the care and use of laboratory animals and the European Union Directive 2010/63/EU on the housing, care, and use of laboratory animals (European Union 2010).

## Oral Acute Toxicity

Acute toxicity evaluation was performed according to OECD guideline No. 423 (OECD 2001). Female mice were randomly divided into groups of three animals per lot (n = 3) and marked for individual identification. Hydro-ethanolic extracts of male spathes (S-M) and female spathes (*Mech Degla* variety S-MD) were administered orally at two single escalating doses (300 and 2000 mg/kg body weight). Initially, the animals received a 300 mg/kg dose and were monitored for 48 h to detect any clinical signs of toxicity (tremors, salivation, convulsions, diarrhea, changes in locomotor activity, loss of appetite, respiratory difficulty, general weakness, or coma) as well as mortality. In the absence of clinical manifestations, the dose was increased to 2000 mg/kg according to the progressive scheme recommended by the OECD. Both doses were administered at a volume of 10 mL/kg. The control group received the same volume of distilled water. Mice in experimental groups were closely observed compared to the control group during the first 4 h, periodically for 48 h, and then daily for 14 days to detect any mortality, clinical signs, or behavioral changes. Body weights were recorded weekly.

## Oral Subacute Toxicity

The subacute study of S-M and S-MD extracts was conducted according to OECD guideline No. 407 (OECD 2008). Male and female mice were randomly divided into seven groups of eight animals each, including four males and four females. The experimental groups were orally administered the two extracts daily at escalating doses of 50, 300, and 1000 mg/kg body weight using a fixed volume of 10 mL/kg for 28 consecutive days. The control group received distilled water. Signs and symptoms of toxicity were observed regularly for 48 h and then once daily for 28 days to detect clinical symptoms of toxicity. The body weight of each mouse was measured weekly during the experiment.

## Blood and Organ Collection

At the end of the experimental period of both studies (acute and subacute), blood sampling was performed *via* caudal vein puncture by a qualified veterinarian (Diehl *et al.* 2001). Blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) for hematological analyses and heparinized tubes for biochemical analyses. The mice were then anesthetized until there was an absence of movement and reflex loss, followed by cervical dislocation to ensure death. This euthanasia method ensures a rapid and painless death while minimizing suffering. This procedure was conducted in accordance with Directive 2010/63/EU of the European Parliament and Council of September 22, 2010, on the protection of animals used for scientific purposes, which ensured compliance with established ethical standards. The organs (liver, kidneys, spleen, heart, and brain) were carefully dissected, washed, and weighed for histopathological study and to assess their relative weight (Sellers *et al*. 2007). The relative organ weight of each mouse was calculated using the following equation (Das *et al*. 2015):

$$\text{Relative organ weight (\%)} = 100 \times [\text{absolute organ weight (g)} / \text{body weight (g)}] \quad (1)$$

## Hematological and Biochemical Analyses

The collected samples were sent to the medical analysis laboratory of the Pasteur Institute of Algeria for further analysis. Blood samples in EDTA tubes were immediately analyzed using a hematology analyzer (Orphee Mythic 18, Suisse) to evaluate various hematological parameters including red blood cells (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), lymphocyte (LYM), granulocyte (GRA), monocyte (MON), platelets (PLT), and mean platelet volume (MPV).

For biochemical analyses, blood samples were stored for 2 h to clot and then centrifuged at 3000 rpm for 15 min to separate the serum. Liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as bilirubin, were analyzed to assess liver function. Creatinine and urea levels were measured to evaluate kidney function. Lipid profiles, including total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels, were also assessed to evaluate lipid metabolism. All analyses were performed using an automated chemical analyzer.

## Histopathological Analyses

The target organs were examined macroscopically to detect any visible pathological changes due to exposure to the tested extracts compared to the control groups. Tissue samples were preserved in a 10% formalin solution for 24 to 48h to maintain tissue integrity and allow histopathological examinations. After fixation, the tissues were cut and dehydrated by immersion in alcohol baths of increasing concentrations to remove the water. This step was followed by clarification with xylene to eliminate alcohol and prepare the tissues for embedding. During this process, the samples were placed in cassettes and immersed in molten paraffin in stainless steel molds. After paraffin solidification, the blocks were stored at -20 °C. The embedded tissue blocks were sectioned into thin slices (4 to 5 µm thick) using a rotary microtome (Leica 2125, Germany). The sections were then floated in a water bath at 50 °C to flatten them and mounted onto glass microscope slides (Thammitiyagodage *et al*. 2020). The microscope slides were dried and stained with hematoxylin and eosin (H&E) to visualize cellular structures and tissue architecture.

Hematoxylin stains cell nuclei black-blue, showing good intranuclear detail, whereas eosin stains the cell cytoplasm and most connective tissue fibers in varying shades and intensities of pink, orange, and red (Alam and Ian 1996). After staining, the slides were covered with a mounting medium to preserve the stained tissue sections and protect them from further damage. The stained sections were observed using an optical microscope equipped with a digital camera (16.1 megapixels), and images were recorded.

### Statistical Analysis

Data analysis and graphs were performed using GraphPad Prism (version 10.5.0, USA). The results are expressed as the mean and standard deviation (mean± SD), and the difference between groups was calculated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. Differences were considered significant at $P < 0.05$. Regarding the phytochemical screening, the variations showed statistically significant differences between the tested extracts ($P < 0.05$). In contrast, there are no significant differences in toxicity parameters between the treatment and the control groups ($P > 0.05$).

## RESULTS AND DISCUSSION

### Extraction Yield

Table 1 presents the results related to the extraction yield, TPC, and TFC. The results obtained in this study highlight the notable extraction efficiency and abundance of these bioactive compounds in both extracts. These values are consistent with those obtained in previous study reporting high polyphenol and flavonoid content in date palm spathes (Farboodniay *et al.* 2016; Asadi *et al*. 2025). These results suggest that date palm spathes are a major source of bioactive metabolites.

**Table 1.** Extraction Yield, Phenolic and Flavonoid Contents of Date Palm Spathes Extracts

| Extracts | Yield (%) | TPC (mg GAE/$g_{extract}$) | TFC (mg QE/$g_{extract}$) | P values |
|---|---|---|---|---|
| **S-MD** | 20.41 ± 0.86 | 335.06 ± 3.52$^{(a)}$ | 260.97 ± 2.86$^{(a)}$ | <0.0001 |
| **S-M** | 17.76 ± 1.27 | 275.59 ± 3.60$^{(b)}$ | 187.46 ± 3.62$^{(b)}$ | <0.0001 |

Note: Data are expressed as mean ±SD (n=3): (a) and (b) significantly different ($P < 0.05$).

### Oral Acute Toxicity

*Clinical observation and mortality*

Single oral administration of S-M and S-MD extracts at doses of 300 and 2000 mg/kg did not cause any clinical signs of toxicity or notable behavioral changes in mice during the 14-day observation period. Moreover, no mortality was recorded in any of the experimental groups, including at the maximum dose of 2000 mg/kg. To date, the LD50 values of phenolic extracts from spathes are not available in the literature. Therefore, it is necessary to examine the potential safety of these compounds and determine the appropriate dose. The present results indicate that these extracts are relatively safe, with LD50 values greater than 2000 mg/kg. This result aligns with previous research on the methanolic pollen extract of date palm, which also showed that at the same dose, no toxic manifestations or mortality were observed, indicating LD50 values greater than 2000

mg/kg (Abdelrahim and Ahmed 2024). Furthermore, a previous study documented the LD50 of date palm seed essential oil as greater than 2000 mg/kg (Oluyele *et al*. 2022). Similarly, the administration of Libyan pollen extract showed no toxic effects up to 5000 mg/kg, confirming the low toxicity of this species (Ashour *et al*. 2022). According to the Hodge and Sterner classification (Lu 1992) and the Globally Harmonized System (GHS) (United Nations 2002), the S-M and S-MD extracts are slightly toxic, providing a good safety margin for the use of date palm residues.

*Body weight variation*

The assessment of mice body weight during the experimental period is shown in Figs. 1a and 1b. All treated groups, regardless of the dose or extract administered, exhibited a regular increase in body weight comparable to that observed in the control group, with no statistically significant differences ($P > 0.05$). These results indicate that the treatment did not affect mouse growth, reflecting the absence of detectable toxic effects on body weight at the administered dose. Similar observations were reported by Bedan *et al*. (2023), who showed that oral administration of aqueous date palm pollen extract caused no weight alterations up to 2900 mg/kg. In contrast, slight weight loss was observed at 5000 mg/kg, which was attributed to temporary reduction in appetite. In a study on the aqueous extract of *Phoenix dactylifera* (L.) seeds, normal body weight gain was observed, and no external abnormalities were seen in any animal at doses up to 5 g/kg (Fakhri *et al*. 2018).

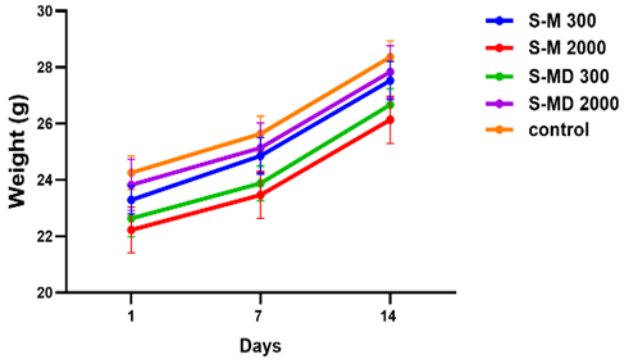



**Fig. 1a.** Effects of acute oral administration of date palm spathes extracts on the body weights of female mice, mean ± SD, n = 3 ($P > 0.05$)

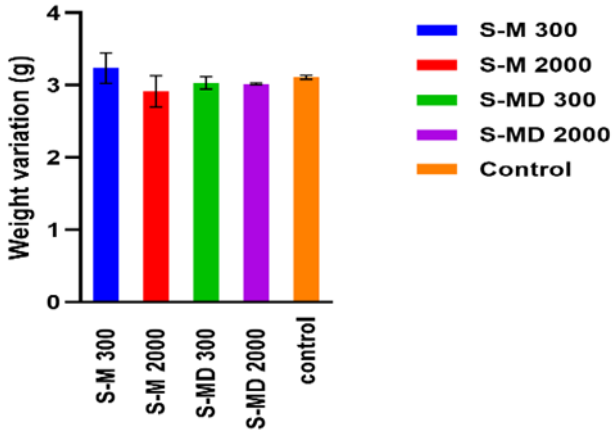



**Fig. 1b.** Effects of acute oral administration of date palm spathes extracts on body weight gain of female mice, mean ± SD, n = 3 ($P > 0.05$)

*Relative organ weight*

The evaluation of relative organ weight is an essential indicator in toxicity studies and a sensitive criterion for potential substance safety, as it can reveal organ changes, even in the absence of clinical symptoms. This approach complements or confirms biochemical, hematological, and histopathological observations (Sellers *et al*. 2007). In the present acute study, no significant variation in relative organ weight was observed between mice treated with 300 and 2000 mg/kg doses and those in the control group (Fig. 2). The recorded values remained comparable and proportional to animal weight, indicating that the treatment did not cause hypertrophy or atrophy of target organs. A previous study showed that administration of date powder extract did not cause changes in the studied organs at doses up to 0.5 g/kg (Sohail *et al*. 2018).

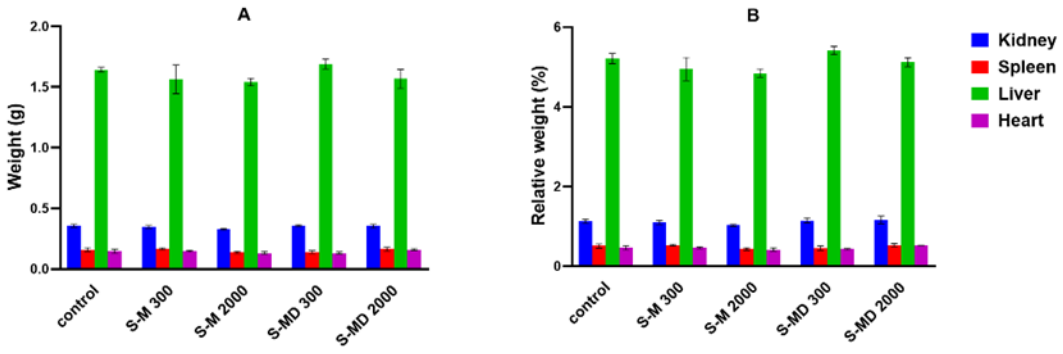



**Fig. 2.** Effects of acute oral administration of date palm spathes extracts on the organ (A) and relative organ weights (B) of female mice, mean ± SD, n = 3 (P > 0.05)

*Biochemical and hematological parameters*

Table 2 presents the results for the biochemical parameters. Evaluation of the liver and kidney parameters showed no notable variations between the treated and control groups (P > 0.05). Serum AST, ALT, and bilirubin levels remained within normal physiological values, with no significant difference compared to the control. Similarly, the urea and creatinine concentrations did not show any significant changes. Regarding metabolic parameters, triglyceride and cholesterol levels were similar between the control and treated groups.

**Table 2.** Effects of Acute Oral Administration of Date Palm Spathes Extracts on the Biochemical Parameters of Female Mice

| | Control Group | S-M 300 mg/kg | S-M 2000 mg/kg | S-MD 300 mg/kg | S-MD 2000 mg/kg |
|---|---|---|---|---|---|
| **ALT (U/L)** | 26.00 ± 4.72 | 28.67 ± 1.76 | 21.00 ± 1.16 | 23.67 ± 1.45 | 21.33 ± 1.76 |
| **AST (U/L)** | 141.0 ± 17.1 | 141.0 ± 22.2 | 137.0 ± 18.7 | 103.3 ± 7.3 | 131.0 ± 5.5 |
| **Bilirubin (mg/L)** | 2.093 ± 0.177 | 1.683 ± 0.277 | 2.043 ± 0.259 | 2.153 ± 0.217 | 1.920 ± 0.322 |
| **Urea (g/L)** | 0.277 ± 0.027 | 0.250 ± 0.021 | 0.223 ± 0.024 | 0.213 ± 0.026 | 0.180 ± 0.032 |
| **Creatinine (mg/L)** | 0.757± 0.137 | 0.767± 0.193 | 0.743 ± 0.200 | 0.813 ± 0.158 | 0.950 ± 0.046 |
| **Triglyceride (g/L)** | 2.307 ± 0.215 | 2.070 ± 0.277 | 2.027 ± 0.237 | 2.080 ± 0.176 | 2.153 ± 0.252 |
| **Cholesterol (g/L)** | 0.990 ± 0.158 | 0.897 ± 0.136 | 0.830 ± 0.160 | 0.796 ± 0.118 | 0.813 ± 0.111 |

Note: Data are expressed as mean ± SD (n = 3); P > 0.05 (not significant).

The evaluation of hematological parameters in mice treated with 300 and 2000 mg/kg of the extracts is presented in Table 3. Overall, no indicators of hematologic toxicity were noted. For white blood cells (WBC), the group treated with 2000 mg/kg of S-MD extract showed a non-significant increase compared to the control, while the other groups remained close to the control values. Red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), and platelet (PLT) values remained comparable to those of the control group. Similarly, lymphocyte (LYM), granulocyte (GRA), and monocyte (Mon) percentages showed no notable variations between the treated groups and the control. Finally, erythrocyte indices, such as mean corpuscular volume (MCV), mean platelet volume (MPV), and mean corpuscular hemoglobin concentration (MCHC), revealed no significant differences.

**Table 3.** Effects of Acute Oral Administration of Date Palm Spathes Extracts on the Hematological Parameters of Female Mice

| | Control Group | S-M 300 mg/kg | S-M 2000 mg/kg | S-MD 300 mg/kg | S-MD 2000 mg/kg |
|---|---|---|---|---|---|
| **WBC ($10^3$/µL)** | 2.667 ± 0.120 | 2.467 ± 0.176 | 2.567 ± 0.176 | 2.533 ± 0.088 | 2.733 ± 0.176 |
| **Lym (%)** | 88.97 ± 1.38 | 85.07 ± 1.12 | 85.60 ± 2.78 | 86.07 ± 2.25 | 85.60 ± 1.33 |
| **GRA (%)** | 10.53 ± 0.49 | 10.07 ± 0.20 | 10.53 ± 0.58 | 10.53 ± 0.71 | 11.17 ± 0.18 |
| **MON (%)** | 5.533 ± 0.203 | 5.167 ± 0.202 | 5.267 ± 0.233 | 5.067 ± 0.273 | 5.200 ± 0.153 |
| **RBC ($10^6$/µL)** | 3.470 ± 0.042 | 3.277 ± 0.061 | 3.430 ± 0.072 | 3.283 ± 0.035 | 3.407 ± 0.076 |
| **Hb (g/dL)** | 4.567 ± 0.176 | 4.333 ± 0.088 | 4.500 ± 0.208 | 4.700 ± 0.265 | 4.300 ± 0.115 |
| **HCT (%)** | 14.57± 0.33 | 14.20 ± 0.17 | 14.80 ± 0.23 | 14.60 ± 0.40 | 14.30 ± 0.21 |
| **MCV (µm$^3$)** | 41.83 ± 0.52 | 41.50 ± 0.40 | 42.27 ± 0.70 | 41.43 ± 0.75 | 42.57 ± 0.34 |
| **MCHC (g/dL)** | 31.90 ± 0.46 | 32.20 ± 0.26 | 31.80 ± 0.17 | 31.90 ± 0.25 | 31.97 ± 0.74 |
| **PLT ($10^3$/µL)** | 446.7 ± 4.1 | 446.7 ± 11.8 | 452.0 ± 6.1 | 468.3 ± 7.3 | 440.0 ± 10.2 |
| **MPV (µm$^3$)** | 6.233 ± 0.240 | 6.033 ± 0.240 | 6.100 ± 0.153 | 5.900 ± 0.379 | 6.300 ± 0.115 |

Note: Data are expressed as mean ± SD (n = 3); P > 0.05 (not significant).

The impact of an extract administered to experimental animals was assessed through hematological and biochemical analyses, which are essential indicators of physiological disturbances and provide predictive information on toxicity. Toxic doses can cause changes in hematological constituents, making it possible to diagnose the adverse effects of foreign substances in the body. In addition, biochemical analysis of serum is essential for determining the functional integrity of organs (Arika and Nyamai 2016; Husic-Selimovic *et al*. 2021). This absence of modification in the present results suggests that the extracts did not alter liver, kidney, or hematopoietic functions, even at the highest dose of 2000 mg/kg. Consequently, these extracts did not induce harmful effects on overall metabolism or systemic disturbances that were detectable in the short term. Previous studies have reported similar observations for various extracts of *B. Coctatum* stem bark, which showed no statistically significant differences in biochemical and hematological parameters at a dose of 5000 mg/kg (Bello *et al*. 2022). Similarly, a study on the methanolic leaf extract of *Guiera senegalensis* noted only slight enzymatic variations (decrease in alkaline phosphatase and increase in AST and) at high doses, without histological damage, confirming that these changes are often reversible (Ahmed *et al*. 2022).

## Oral Subacute Toxicity

*Clinical observation and mortality*

The subacute toxicity study of S-M and S-MD polyphenolic spathe extracts of *Phoenix dactylifera* (L.) aimed to determine the cumulative or progressive effects of repeated exposure over a prolonged period (28 days). The study showed that daily administration of the tested extracts at doses of 50, 300, and 1000 mg/kg revealed no clinical signs of toxicity or mortality throughout the experimental period, suggesting the non-toxicity of these extracts over an extended period of time.

*Body weight variation*

During the 28-day experiment, repeated oral administration of the tested extracts did not induce any significant differences in body weight, either based on the different doses administered or by sex. All experimental groups showed regular weight gain over the weeks (Figs. 3a and 3b). The absence of significant variations in body weight suggests that the extracts do not disrupt body weight gain, regardless of sex. This indicates that the treatment did not affect the appetite and food intake in mice, consistent with the observations reported by Abdelrahim and Ahmed (2024), who showed that repeated administration of methanolic pollen extract of *Phoenix dactylifera* for 28 days caused no significant toxic effects on body weight in both sexes. Oluyele *et al*. (2022) reported normal weight gain in mice and the absence of clinical signs of toxicity.

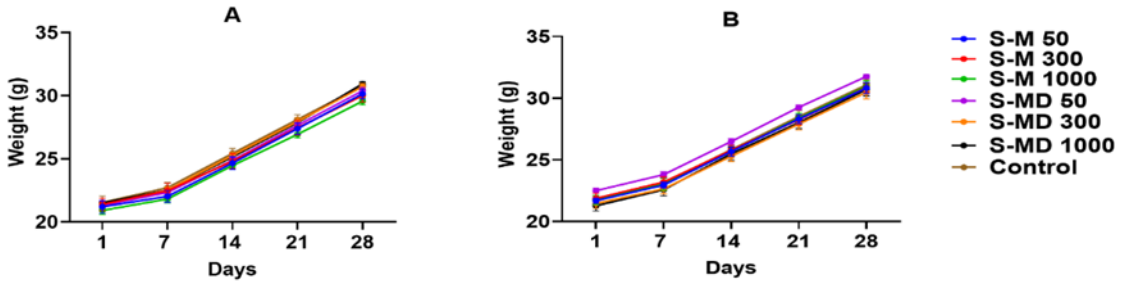



**Fig. 3a.** Effects of subacute oral administration of date palm spathes extracts on the body weights of female (A) and male (B) mice, mean ± SD, n = 4 (P > 0.05)

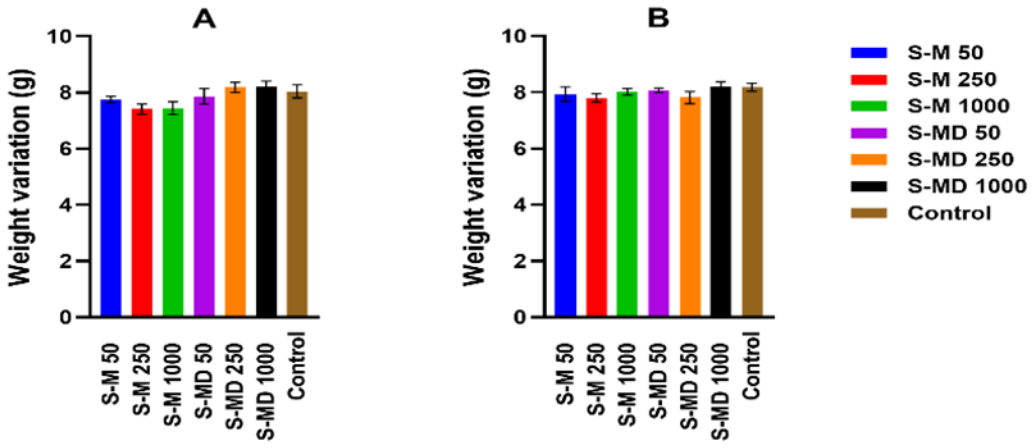



**Fig. 3b.** Effects of subacute oral administration of date palm spathes extracts on body weight gain of female (A) and male (B) mice, mean ± SD, n = 4 (P > 0.05)

*Relative organ weight*

Evaluation of the relative weights of major organs, including the kidneys, liver, spleen, heart, and brain, showed no statistically significant variations between the treated and the control groups in both male and female mice (Figs. 4 and 5). These data indicate that daily administration of the extracts at doses of 50, 300, and 1000 mg/kg did not adversely affect general metabolic status and did not exert cumulative toxic effects on vital organs. Similar observations have been reported in previous studies by Oluyele *et al*. (2022) and Abdelrahim and Ahmed (2024), showing no significant changes in the relative weights.

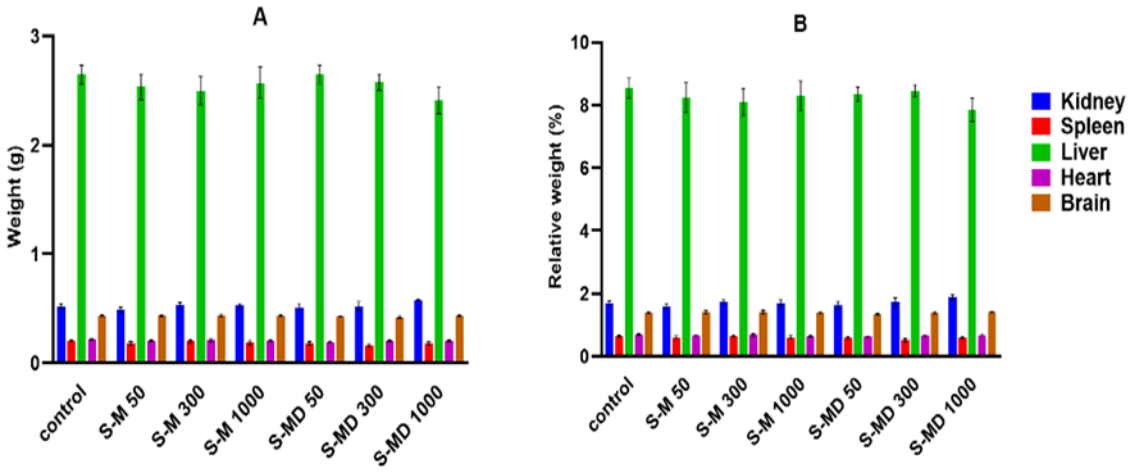



**Fig. 4.** Effects of subacute oral administration of date palm spathes extracts on the organ (A) and relative organ weights (B) of female mice, mean ± SD, n=4 (P > 0.05)

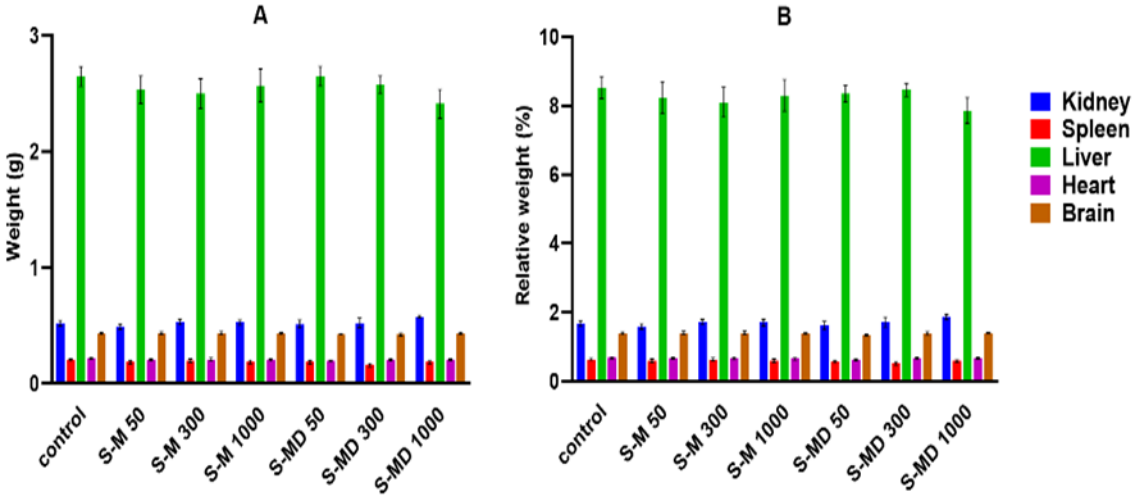



**Fig. 5.** Effects of subacute oral administration of date palm spathes extracts on the organ (A) and relative organ weights (B) of male mice, mean ± SD, n=4 (P > 0.05)

**Table 4.** Effects of Subacute Oral Administration of Date Palm Spathes Extracts on the Biochemical Parameters of Female and Male Mice

| | | Control Group | S-M 50 mg/kg | S-M 300 mg/Kg | S-M 1000 mg/kg | S-MD 50 mg/kg | S-MD 300 mg/kg | S-MD 1000 mg/kg |
|---|---|---|---|---|---|---|---|---|
| ALT (U/L) | Female | 25.75 ± 3.64 | 21.50 ± 1.85 | 21.50 ± 1.32 | 23.25 ± 2.50 | 22.00 ± 1.29 | 21.75 ± 2.59 | 21.75 ± 2.59 |
| | Male | 39.75 ± 4.99 | 30.00 ± 4.74 | 32.50 ± 6.38 | 35.25 ± 6.54 | 29.25 ± 4.89 | 31.00 ± 4.60 | 31.00 ± 4.60 |
| AST (U/L) | Female | 153.5 ± 17.4 | 120.8 ± 7.3 | 139.0 ± 13.2 | 154.0 ± 14.4 | 149.5 ± 14.8 | 127.8 ± 8.1 | 127.8 ± 8.1 |
| | Male | 185.5 ± 8.3 | 180.8 ± 10.9 | 143.0 ± 9.8 | 156.8 ± 19.9 | 142.3 ± 17.5 | 167.5 ± 19.9 | 167.5 ± 19.9 |
| Bilirubin (mg/L) | Female | 2.05 ± 0.14 | 1.84 ± 0.10 | 1.95 ± 0.14 | 2.16 ± 0.09 | 1.91 ± 0.17 | 2.00 ± 0.18 | 2.00 ± 0.18 |
| | Male | 2.19 ± 0.04 | 2.08 ± 0.07 | 2.19 ± 0.03 | 2.14 ± 0.08 | 2.18 ± 0.04 | 2.12 ± 0.10 | 2.12 ± 0.10 |
| Urea (g/L) | Female | 0.21 ± 0.01 | 0.21 ± 0.01 | 0.23 ± 0.02 | 0.22 ± 0.02 | 0.21 ± 0.01 | 0.21 ± 0.02 | 0.21 ± 0.02 |
| | Male | 0.19 ± 0.01 | 0.16 ± 0.01 | 0.18 ± 0.01 | 0.18 ± 0.02 | 0.19 ± 0.01 | 0.16 ± 0.01 | 0.16 ± 0.01 |
| Creatinine (mg/L) | Female | 1.32 ± 0.06 | 1.31 ± 0.04 | 1.34 ± 0.04 | 1.34 ± 0.04 | 1.34 ± 0.07 | 1.32 ± 0.04 | 1.32 ± 0.04 |
| | Male | 1.50 ± 0.04 | 1.54 ± 0.04 | 1.54 ± 0.04 | 1.54 ± 0.04 | 1.51 ± 0.06 | 1.52 ± 0.04 | 1.52 ± 0.04 |
| Triglycerides (g/L) | Female | 0.67 ± 0.06 | 0.58 ± 0.03 | 0.59 ± 0.06 | 0.59 ± 0.03 | 0.62 ± 0.04 | 0.58 ± 0.04 | 0.58 ± 0.04 |
| | Male | 0.71 ± 0.06 | 0.61 ± 0.11 | 0.71 ± 0.15 | 0.71 ± 0.07 | 0.68 ± 0.11 | 0.66 ± 0.04 | 0.66 ± 0.04 |
| Cholesterol (g/L) | Female | 0.54 ± 0.03 | 0.55 ± 0.04 | 0.57 ± 0.02 | 0.52 ± 0.01 | 0.56 ± 0.04 | 0.54 ± 0.03 | 0.54 ± 0.03 |
| | Male | 0.54 ± 0.02 | 0.57 ± 0.05 | 0.54 ± 0.06 | 0.58 ± 0.04 | 0.59 ± 0.02 | 0.51 ± 0.02 | 0.51 ± 0.02 |
| HDL (g/L) | Female | 0.22 ± 0.01 | 0.24 ± 0.01 | 0.21 ± 0.01 | 0.21 ± 0.01 | 0.22 ± 0.02 | 0.22 ± 0.01 | 0.22 ± 0.01 |
| | Male | 0.22 ± 0.01 | 0.25 ± 0.03 | 0.22 ± 0.03 | 0.23 ± 0.02 | 0.28 ± 0.02 | 0.22 ± 0.01 | 0.22 ± 0.01 |
| LDL (g/L) | Female | 0.18 ± 0.01 | 0.19 ± 0.04 | 0.22 ± 0.03 | 0.19 ± 0.02 | 0.21 ± 0.03 | 0.20 ± 0.03 | 0.20 ± 0.03 |
| | Male | 0.18 ± 0.01 | 0.20 ± 0.02 | 0.18 ± 0.02 | 0.20 ± 0.01 | 0.17 ± 0.02 | 0.16 ± 0.02 | 0.16 ± 0.02 |

Note: Data are expressed as mean ± SD (n=4). $P > 0.05$ (not significant).

*Biochemical and hematological parameters*

Repeated oral administration of S-M and S-MD extracts for 28 days did not cause any notable biochemical changes in mice, regardless of sex. Hepatic biomarkers, including ALT, AST, and bilirubin as well as renal biomarkers, including creatinine and urea, remained within physiological limits, excluding any hepatic or renal damage. Additionally, the evaluation of parameters associated with bile and lipid metabolism (triglycerides, total cholesterol, LDL, and HDL) showed results similar to those of the control group (Table 4).

As shown in Table 5, in both male and female mice, hematological evaluation showed no significant differences between the treated groups and controls ($P > 0.05$). The globular indices WBC, RBC, Hb, HCT, MCV, and MCHC, as well as the platelets and leukocyte subgroups, including LYM, MON, and GRA, remained within normal physiological limits.

**Table 5.** Effects of Subacute Oral Administration of Date Palm Spathes Extracts on the Hematological Parameters of Female and Male Mice

| | | Control Group | S-M 50 mg/kg | S-M 300 mg/kg | S-M 1000 mg/kg | S-MD 50 mg/kg | S-MD 300 mg/kg | S-MD 1000 mg/kg |
|---|---|---|---|---|---|---|---|---|
| WBC ($10^3$/µL) | Female | 3.20 ± 0.37 | 2.75 ± 0.20 | 2.65 ± 0.30 | 2.82 ± 0.14 | 3.10 ± 0.38 | 2.88 ± 0.34 | 2.88 ± 0.34 |
| | male | 3.98 ± 0.36 | 3.52 ± 0.25 | 3.80 ± 0.18 | 3.58 ± 0.29 | 3.88 ± 0.41 | 3.65 ± 0.22 | 3.65 ± 0.22 |
| Lym (%) | Female | 89.03 ± 1.70 | 86.10 ± 1.32 | 84.38 ± 0.97 | 84.73 ± 1.23 | 86.73 ± 2.00 | 88.10 ± 0.66 | 88.10 ± 0.66 |
| | Male | 90.55 ± 1.20 | 89.13 ± 1.90 | 89.18 ± 1.47 | 86.15 ± 1.33 | 89.95 ± 2.33 | 89.30 ± 2.20 | 89.30 ± 2.20 |
| GRA (%) | Female | 10.80 ± 0.38 | 10.43 ± 0.45 | 10.38 ± 0.34 | 10.38 ± 0.48 | 10.70 ± 0.18 | 10.38 ± 0.32 | 10.38 ± 0.32 |
| | Male | 9.62 ± 0.36 | 9.22 ± 0.35 | 9.52 ± 0.42 | 9.72 ± 0.40 | 9.40 ± 0.27 | 9.62 ± 0.40 | 9.62 ± 0.40 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **MON (%)** | Female | 5.42 ± 0.25 | 5.02 ± 0.22 | 5.15 ± 0.17 | 5.22 ± 0.16 | 5.10 ± 0.26 | 4.78 ± 0.12 | 4.78 ± 0.12 |
| | Male | 3.82 ± 0.39 | 3.72 ± 0.24 | 3.82 ± 0.28 | 3.95 ± 0.23 | 4.02 ± 0.25 | 3.72 ± 0.23 | 3.72 ± 0.23 |
| **RBC ($10^6$/µL)** | Female | 3.33 ± 0.21 | 3.41 ± 0.13 | 3.41 ± 0.08 | 3.70 ± 0.16 | 3.38 ± 0.12 | 3.48 ± 0.11 | 3.48 ± 0.11 |
| | Male | 3.36 ± 0.10 | 3.22 ± 0.15 | 3.32 ± 0.12 | 3.40 ± 0.17 | 3.33 ± 0.19 | 3.33 ± 0.09 | 3.33 ± 0.09 |
| **Hb (g/dL)** | Female | 5.12 ± 0.21 | 4.72 ± 0.20 | 4.98 ± 0.29 | 5.15 ± 0.21 | 4.85 ± 0.33 | 5.12 ± 0.18 | 5.12 ± 0.18 |
| | Male | 4.40 ± 0.25 | 4.30 ± 0.19 | 4.05 ± 0.23 | 4.18 ± 0.17 | 4.05 ± 0.17 | 4.20 ± 0.24 | 4.20 ± 0.24 |
| **HCT (%)** | Female | 15.18 ± 0.58 | 15.35 ± 0.51 | 15.55 ± 0.48 | 15.13 ± 0.46 | 15.68 ± 0.29 | 15.35 ± 0.44 | 15.35 ± 0.44 |
| | Male | 14.83 ± 0.56 | 14.28 ± 0.28 | 14.28 ± 0.30 | 14.55 ± 0.51 | 14.20 ± 0.21 | 14.18 ± 0.51 | 14.18 ± 0.51 |
| **MCV ($\mu m^3$)** | Female | 45.08 ± 0.62 | 44.65 ± 0.50 | 44.13 ± 0.54 | 43.53 ± 0.38 | 45.13 ± 0.69 | 44.48 ± 0.91 | 44.48 ± 0.91 |
| | Male | 44.43 ± 0.76 | 43.95 ± 0.48 | 42.70 ± 1.15 | 44.20 ± 0.38 | 43.95 ± 0.79 | 43.50 ± 0.79 | 43.50 ± 0.79 |
| **MCHC (g/dL)** | Female | 31.03 ± 0.62 | 29.90 ± 0.66 | 30.18 ± 0.43 | 31.10 ± 0.60 | 30.55 ± 0.43 | 31.00 ± 0.45 | 31.00 ± 0.45 |
| | Male | 30.58 ± 0.48 | 30.43 ± 0.50 | 30.33 ± 0.57 | 30.85 ± 0.61 | 30.13 ± 0.48 | 30.53 ± 0.38 | 30.53 ± 0.38 |
| **PLT ($10^3$/µL)** | Female | 516.8 ± 12.2 | 471.8 ± 14.9 | 510.5 ± 6.9 | 515.5 ± 14.6 | 499.5 ± 15.8 | 473.5 ± 27.7 | 473.5 ± 27.7 |
| | Male | 553.8 ± 33.3 | 562.3 ± 16.7 | 583.5 ± 13.5 | 530.8 ± 36.5 | 518.0 ± 11.8 | 544.5 ± 29.4 | 544.5 ± 29.4 |
| **MPV ($\mu m^3$)** | Female | 5.98 ± 0.27 | 5.85 ± 0.24 | 5.925 ± 0.239 | 6.02 ± 0.25 | 5.98 ± 0.14 | 5.75 ± 0.26 | 5.75 ± 0.26 |
| | Male | 5.98 ± 0.18 | 6.12 ± 0.16 | 6.225 ± 0.320 | 6.22 ± 0.18 | 5.85 ± 0.14 | 5.90 ± 0.19 | 5.90 ± 0.19 |

Note: Data are expressed as mean ± SD (n=4). P > 0.05 (not significant)

In the subacute toxicity study, the absence of significant alterations in serum biochemical markers indicated the preservation of functional integrity of major organs. Similarly, the stability of hematological parameters suggests that the extracts did not impair hematopoietic function or induce hematotoxic, inflammatory, or immune-related responses. These results suggest good long-term metabolic tolerance to the extracts. Similar findings were reported by Abdelrahim and Ahmed (2024) for date palm pollen, Fakhri *et al*. (2018) for seeds, and Oluyele *et al*. (2022) for kernel essential oil, confirming the high safety margin of products from this species. A study on the phenolic fraction of seeds showed no significant variation in hematological or biochemical parameters (Bikri *et al*. 2021).

*Histopathology*

The results of the histopathological examination concerning acute and subacute toxicity in mice, shown in Figs. A.1, A.2, and A.3, revealed an appearance compatible with a normal structure without pathological changes in organ architecture, with some mild, nonspecific lesions. Mild congestion was observed in the heart and kidney. Histopathological examination of liver sections revealed hepatocyte vacuolization, associated with mild congestion. Extramedullary hematopoiesis (EMH) was observed in the spleens. No characteristic lesions were observed in the brains. These findings, which are of low severity, are common and are often associated with metabolic changes or a non-toxic physiological response. These are considered background lesions that are frequently encountered in mice. Given the identical histological profiles observed for both S-M and S-MD extracts across all tested doses, representative micrographs of the control groups and those that received the higher dose (2000 mg/kg for acute toxicity; 1000 mg/kg for subacute toxicity) are presented regardless of the type of extract avoiding data redundancy.

Histopathological analysis of tissues, a crucial element in toxicity studies, allows for the direct observation of tissue architecture to differentiate treatment-specific lesions from physiological and pathological variations, artifacts or background lesions. This provides additional evidence to support the hematological and biochemical results (Sills *et*

*al*. 2019). Photomicrographs of organ sections from mice treated by oral gavage with different doses of S-MD and S-M spathe extracts show minor histological alterations that are considered non-specific in the absence of functional disturbances and clinical signs of disease. These alterations are generally compatible with background lesions commonly encountered in laboratory mice and do not constitute evidence of the toxic effects of the extracts (Mackie 2012). Extramedullary splenic hematopoiesis is a physiological phenomenon frequently recognized as normal in adult mice, indicating the physiological activity of lymphoid tissue rather than a toxic effect in the absence of hematological abnormalities (Cenariu *et al*. 2012). According to the INHAND guidelines, hepatocyte vacuolization and mild congestion in the liver, kidneys, and heart are within the range of changes considered non-proliferative lesions observed in experimental mice, often associated with metabolic variations, stress, or fixation artifacts (Thoolen *et al*. 2010). Minor alterations are generally considered to have no toxicological significance if not accompanied by specific cellular lesions, necrosis, severe congestion, hemorrhage, marked inflammation, or biochemical alterations. Notably, no characteristic brain lesions were observed in the brains of the treated mice, confirming the absence of detectable neuropathological effects, consistent with the lack of observable neurotoxic effects in histology. These results are consistent with those of Bedan *et al*. (2023) and Oluyele *et al*. (2022), who observed no pathological changes in the tested organs.

Overall, the various observations from this study, combined with the total absence of abnormalities in biochemical and hematological parameters and the absence of dose-dependent relationships, strongly suggest that the observed alterations mainly reflect spontaneous or physiological variations rather than adverse toxicity related to the administration of the tested extracts. The absence of histopathological differences related to the extracts indicate that both S-M and S-MD extracts from *Phoenix dactylifera* spathes have a similar safety profile. These extracts are non-toxic and are safe for use under experimental conditions and at the tested doses. This safety can be correlated with the high phenolic and flavonoid contents in these extracts (Table 1). Although the present study did not include detailed phytochemical identification, recent work by Asadi *et al.* (2025) has shown that date palm spathes are rich in total polyphenols, of which caffeic acid is the major phenolic compound. The safety of caffeic acid is well documented: Dekanski *et al.* (2018) reported no acute toxicity in mice after oral administration, and Aijaz *et al.* (2022) confirmed its generally accepted safety profile. Furthermore, Bouhoreira and Dadamoussa (2016) detected flavonoids, including quercetin, in date palm spathes. Consistently, Dibal *et al.* (2020) established an oral LD50 of 3807 mg/kg for quercetin in mice, indicating a wide safety margin. Thus, the the lack of toxicity observed in the present study up to 2000 mg/kg is consistent with the known safety of the main phenolic constituents identified in date palm spathes. Beyond the phytochemical correlations, the choice of the mouse as an experimental model is fundamental to interpret the toxicological relevance of these findings. The absence of toxicity observed in mice suggests a high safety profile for the spathe extract. The mouse is considered a reference model in toxicology, according to OECD guidelines, due to its high physiological and genetic similarity to other mammals, including humans (Perlman 2016). Furthermore, a strong correlation has been established between toxicity in rodents and effects in humans, with studies showing that animal models can predict toxic effects in humans in approximately 70% of cases (Oslon *et al.* 2000). Therefore, this model constitutes a validated first step for evaluating the potential toxicological effects of spathe extracts before considering further research in other biological systems. To reduce the quantitative difference between species and define a safe

therapeutic range, the authors applied body surface area (BSA)-based normalization. For example, the applied safe dose of 2,000 mg/kg in mice corresponds to a human equivalent dose (HED) of approximately 162.2 mg/kg, according to the standard conversion factor (0,081) of Reagan-Shaw *et al.* (2008), providing a comfortable safety margin for future clinical or pharmacological applications of the extract. However, further studies are recommended to confirm this extrapolation.

## CONCLUSIONS

1. In this study, oral administration of ethanolic extracts of the male spathe S-M and female spathe of the *Mech Degla* variety S-MD of *Phoenix dactylifera*, at the tested doses, caused no apparent toxicity, mortality, or notable changes in body weight or organ weight during acute and subacute toxicity tests in NMRI mice.
2. Furthermore, no major variations in the evaluated biochemical and hematological parameters were observed, which remained within the expected physiological values for the murine model. This indicates the absence of disturbances in renal, hepatic, and hematopoietic functions.
3. Histopathological examination of the target organs confirmed the absence of toxicity, highlighting minimal changes corresponding to common background lesions in laboratory rodents, with no dose-dependent relationship or correlation with blood analyses. Collectively, these results indicate that spathe extracts have a wide safety margin and do not generate measurable toxic effects, even at doses of 2000 mg/kg.

### Conflict of Interest

The authors declare no conflict of interest.

### Institutional Review Board Statement

The protocol for this animal study was approved by the Ethics Committee of the Tedjini Damerdji University Hospital Center, Tlemcen (ED.MB.09.25; September 22, 2023). All experiments were conducted in accordance with the internationally accepted principles for the use and care of laboratory animals.

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## APPENDIX

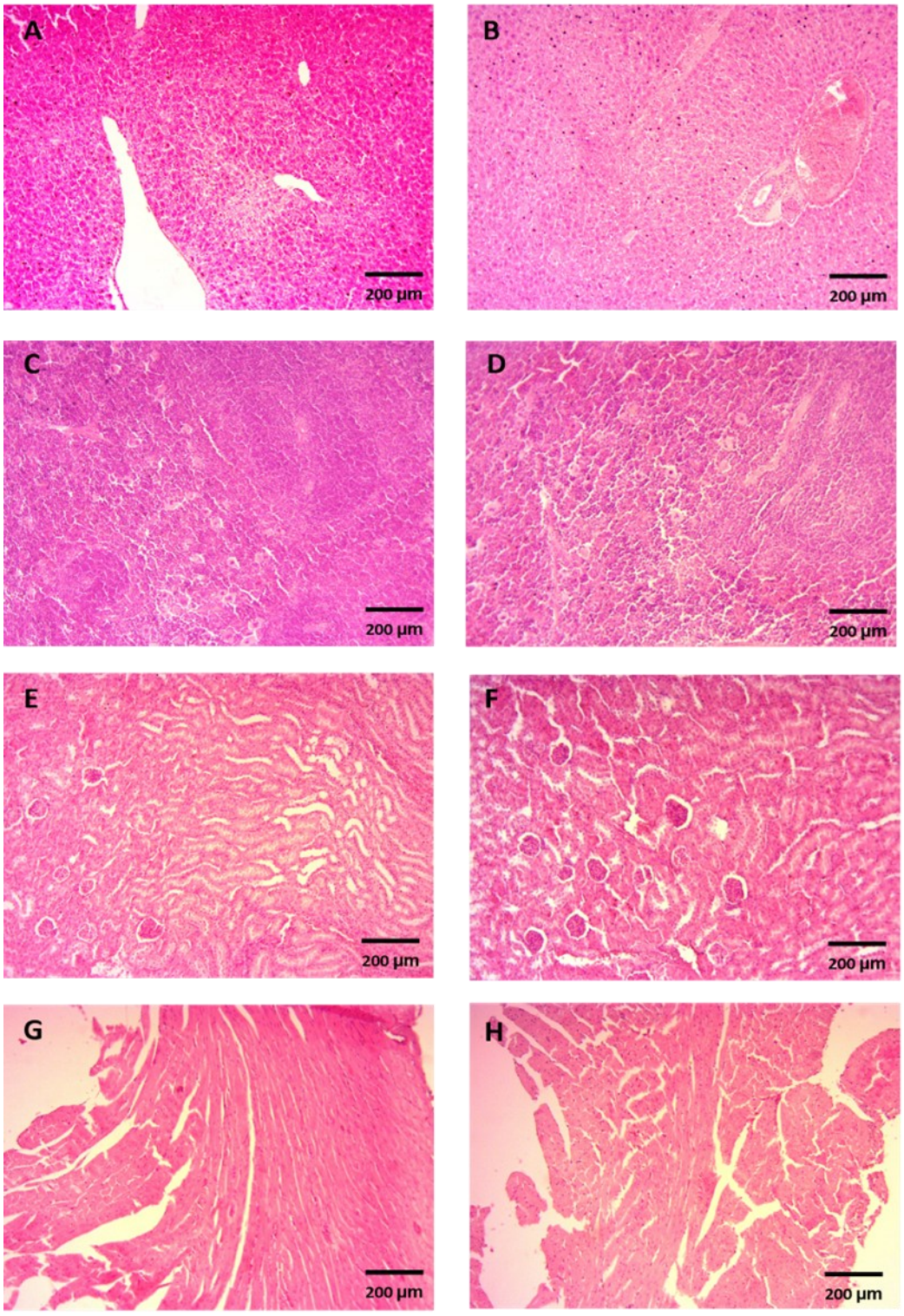



**Fig. A1.** Representative photomicrographs of vital organs following acute oral administration of *Phoenix dactylifera* (L.) spathe extracts. (A) Liver, (C) Spleen, (E) Kidney, (G) Heart from the control group. (B) Liver, (D) Spleen, (F) Kidney, (H) Heart from the treated group with a high dose of 2000 mg/kg. H&E staining x 100

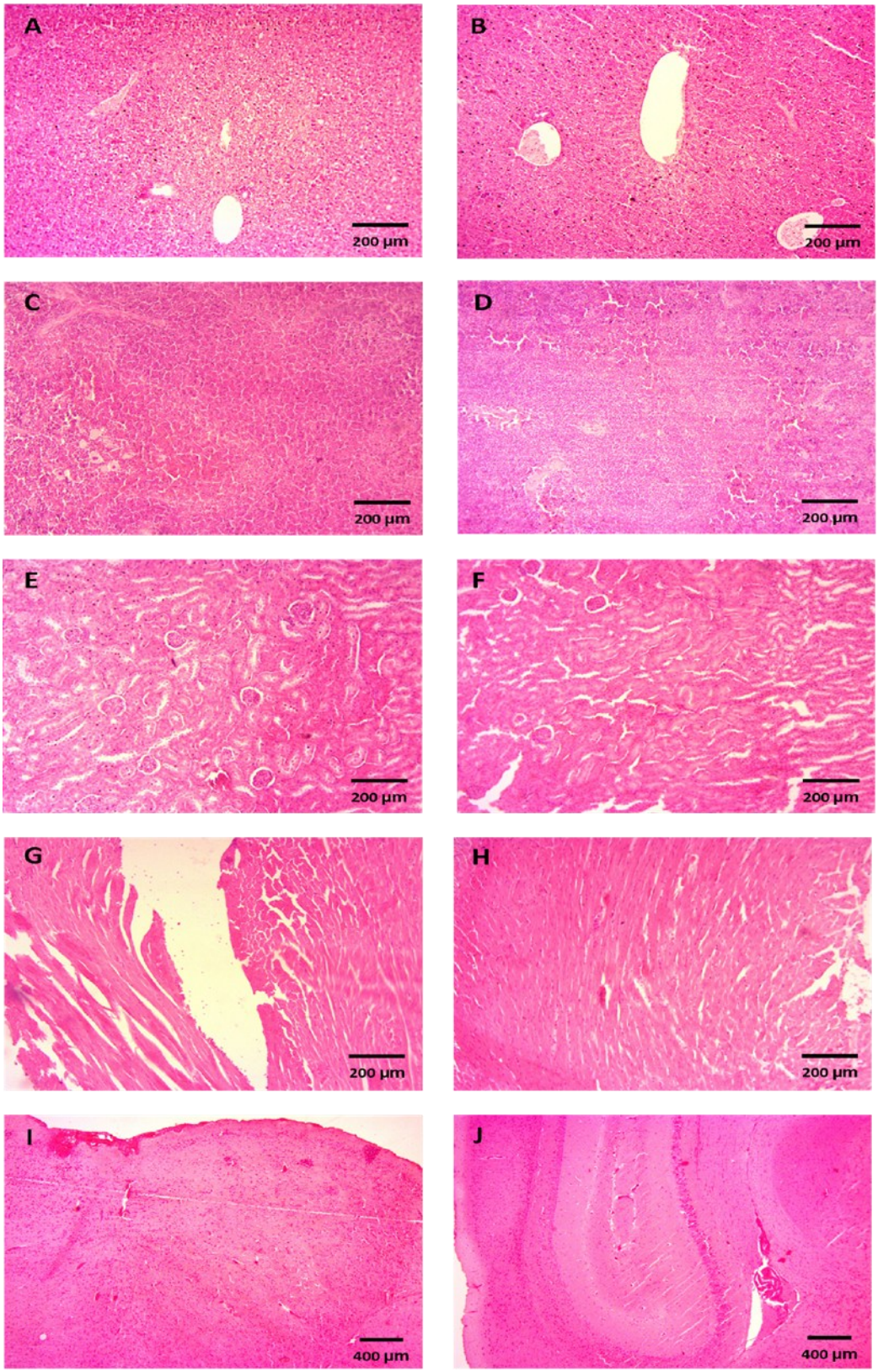



**Fig. A2.** Representative photomicrographs of vital organs from female mice following subacute oral administration of *Phoenix dactylifera* (L.) spathe extracts. (A) Liver, (C) Spleen, (E) Kidney, (G) Heart, (I) Brain from the control group. (B) Liver, (D) Spleen, (F) Kidney, (H) Heart, (J) Brain from the treated group with a high dose of 1000 mg/kg. H&E staining x 100, Brain x40

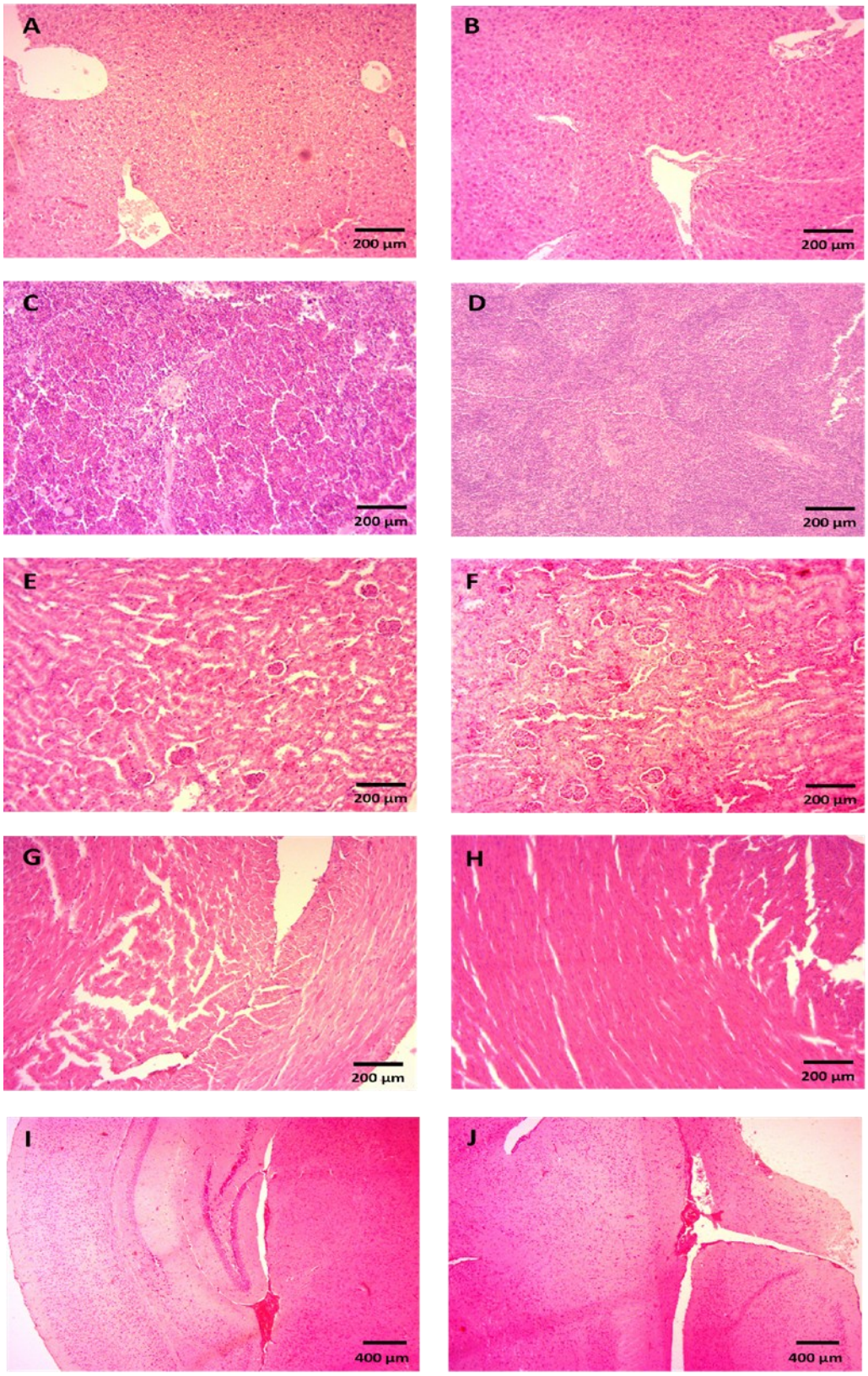



**Fig. A3.** Representative photomicrographs of vital organs from male mice following subacute oral administration of *Phoenix dactylifera* (L.) spathe extracts. (A) Liver, (C) Spleen, (E) Kidney, (G) Heart, (I) Brain from the control group. (B) Liver, (D) Spleen, (F) Kidney, (H) Heart, (J) Brain from the treated group with a high dose of 1000 mg/kg. H&E staining x 100, Brain x40