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## Research Article

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**Toxicological evaluation of seed-derived alkaloids from *Smyrniium olusatrum* L. following acute and repeated intraperitoneal administration in mice**

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## **Abstract**

*Smyrniium olusatrum* L., traditionally used for its medicinal properties, contains alkaloids with various pharmacological activities. Despite its historical use, comprehensive toxicological data remain scarce, limiting its safe integration into modern pharmacological frameworks. This study aimed to assess the acute and subacute toxicity of alkaloids from *S. olusatrum* seeds in Swiss mice.

Acute toxicity was evaluated via intraperitoneal administration of alkaloids at doses of 1, 10, 20, 200, and 2000 mg/kg. Subacute toxicity was assessed through daily intraperitoneal administration of 25, 50, 100, and 200 mg/kg over 28 days. Clinical signs, body and organ weights, hematological and biochemical parameters, and histopathological features were analyzed.

Our results showed no mortality or notable clinical signs observed in the acute phase, suggesting an intraperitoneal LD<sub>50</sub> exceeding 2000 mg/kg. In the subacute study, repeated intraperitoneal administration of alkaloids was well-tolerated, with no significant changes in biochemical or hematological markers. Histopathological evaluation revealed no organ-specific damage, except for mild, dose-dependent, and sex-dependent alterations in the liver of male mice. Alkaloids demonstrate low toxicity in mice. These findings provide a foundational toxicological profile supporting the safe use of *S. olusatrum*-derived bioactive compounds while emphasizing the importance of dose optimization and consideration of sex-specific hepatic responses.

**Keywords:** *Smyrniium olusatrum*; Alkaloids; Liver histopathology, Sex difference.

## Introduction

Natural products have historically served as a foundational source for the discovery and development of medicinal agents (Newman and Cragg 2020). However, many commonly applied empirical rules and drug-likeness filters often fail to account for the structural diversity inherent to natural compounds, particularly alkaloids (Heinrich et al. 2021). Alkaloids are nitrogen-containing, typically basic, cyclic organic molecules known for their structural complexity and broad range of biological activities (Roy 2017). While many alkaloids align with the principles of medicinal chemistry, their unique physicochemical properties often fall outside conventional drug-likeness criteria, which can result in the exclusion of promising bioactive candidates during early stages of drug development (Heinrich et al. 2021).

Indeed, medicinal and aromatic plants constitute one of the main sources of these secondary metabolites. Alkaloids represent approximately 20% of all known compounds in this category (Kaur 2015). These chemical constituents play crucial roles in plant defense mechanisms and have long been recognized for their pharmacological relevance in human medicine (Kaur 2015). Numerous plant-derived alkaloids are used for the treatment of chronic and complex diseases, including cancer, diabetes, and neurological disorders. Their inherent bioactivity originally evolved as a defense strategy against herbivores and microbial pathogens forms the basis for their wide range of pharmacological applications across clinical and industrial domains (Adamski et al. 2020; Heinrich et al. 2021). Moreover, alkaloids exhibit diverse pharmacological effects, including antiviral, antibacterial, anti-inflammatory, and anticancer properties. As a result, significant research efforts continue to focus on their pharmacological properties and the elucidation of their mechanisms of action (Heinrich et al. 2021).

Despite increased scientific interest, the vast diversity of alkaloid structures and sources means that our overall understanding remains incomplete. Therefore, the identification, structural characterization, and quantification of in various medicinal and aromatic plants remain essential to advance this field (Adamski et al. 2020). Furthermore, while many alkaloids offer pharmacological benefits, some have also been associated with toxic effects. Prolonged or excessive exposure to certain alkaloids has been linked to various pathologies, including carcinogenesis (Chang et al. 2019). Evaluating the toxicological profile of bioactive compounds is a crucial step to ensure their safe use and effective pharmacological application.

Despite their pharmacological interest, many medicinal plants remain largely unexplored, both in terms of pharmacology and toxicology. *S. olusatrum*, a member of the Apiaceae family, represents a notable example. Its ethanolic and methanolic extracts, as well as its essential oils, have demonstrated promising pharmacological properties, including antioxidant, anti-inflammatory, and analgesic activities (El Hamsas el Youbi et al., 2024; Sekkout et al., 2024). However, the plant's isolated secondary metabolites, particularly its alkaloids, have received little scientific attention.

Regarding toxicology, available data are very limited. Aside from a single study investigating the acute toxicity of ethanolic and methanolic extracts (El Hamsas el Youbi et al., 2024), no comprehensive studies have evaluated the toxicity of this plant or its bioactive constituents. This lack of information hampers the full pharmacological development and rational use of *S. olusatrum*.

In this context, the present study aims to assess the acute and subacute toxicity of alkaloids extracted from *S. olusatrum*, providing a clearer understanding of their toxicity profile and supporting a more informed and rational use of this plant in pharmacological applications.

## **Materials and methods**

### **Plant material**

Seeds of *S. olusatrum* were obtained from reputable herbalists and identified at the University Hassan I of Settat, receiving the identifier S789I76 (el Hamsas el Youbi et al. 2024). The authenticated specimen was then deposited in the Laboratory of Biology and Health, Faculty of Sciences, Ben M'sik, Casablanca, Morocco.

### **Preparation of *S. olusatrum* extracts**

#### **Alkaloid extraction**

Alkaloid extraction was performed as described by Li et al. (2020). Briefly, thirty grams of dried plant material were extracted with methanol using a Soxhlet apparatus at 40 °C. The resulting methanolic extract was concentrated, acidified, and filtered, then sequentially extracted with hexane and chloroform. The chloroform fractions were combined, concentrated, and the final alkaloid extract was stored at 4 °C until further use.

#### **Phytochemical screening**

Alkaloids were qualitatively detected using Mayer's reagent. As described by Gupta and Patel (2020), the alkaloid extract was treated with 1-2 drops of the reagent, and the appearance of a white precipitate indicated a positive reaction for alkaloids (Gupta and Patel 2020).

#### **Experimental animals**

Male and female mice were obtained from the Tit Mellil *in vivo* experimental center, Casablanca. Animals were maintained under standard laboratory conditions at  $24 \pm 1$  °C with a 12-hour light/dark cycle and had ad libitum access to food and water. All procedures were performed in accordance with international guidelines for the care and use of laboratory animals (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011), and approved by the institutional ethics committee (Approval No. CEEA/2025/001).

#### **Acute toxicity**

A total of 24 mice was given a single intraperitoneal administration of the tested alkaloids in accordance with the experimental protocol outlined in OECD Guideline 423 (OECD 2002). Mice of 29-31g body weight were utilized in the study, and had access to food and water. After acclimatization, the mice were randomly assigned to eleven groups (n = 4 per group; both sexes). The control group received intraperitoneally NaCl 0.9%. Treatment groups A1–A5 received doses of 1, 10, 20, 200, and 2000 mg/kg, respectively. Behavioral and physical changes were monitored throughout the 14-day acute toxicity period. Morbidity was assessed daily, and body weights were recorded weekly. At the end of the 14 days, final body weights were measured, and the animals were sacrificed for organ collection. The relative organ weights were calculated.

#### **Subacute toxicity**

The subacute toxicity of repeated alkaloid administration was evaluated in accordance with the OECD Guideline 407 protocol (OECD 2008). Alkaloids were delivered intraperitoneally at doses of 25, 50, 100 and 200 mg/kg. A total of

seven groups of animals (n = 4 per group; both sexes) were randomly assigned to the study. Six experimental groups received daily intraperitoneal injections of alkaloids dissolved in 0.9% NaCl for a period of 28 days, while the control group was administered 0.9% NaCl alone under the same schedule. Throughout the study duration, animals were monitored daily for any signs of mortality or abnormal clinical symptoms.

### **Biochemical analysis**

Blood samples were collected into red-top tubes for biochemical analyses. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine were quantified using a BS240PRO automated biochemical analyzer. Blood glucose levels were determined using a One Call Plus glucometer. Total cholesterol was measured employing a commercial kit from DiaSys Diagnostic Systems GmbH.

### **Hematological analysis**

Blood samples were collected in EDTA tubes for subsequent hematological evaluation. An automated hematology analyzer (BECKMAN COULTER AU480) was employed to assess a range of hematological parameters. These included white blood cell count (WBC), neutrophil count, lymphocyte count, monocyte count, red blood cell count (RBC), hemoglobin concentration (HB), red blood cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count.

### **Histopathological examination**

Major organs, including the heart, liver, kidneys, lungs, spleen, and brain, were collected from both control and treated animals. The tissues were fixed in 10% formalin, followed by dehydration through a graded ethanol series and clearing with toluene (Brogi et al. 2019). Subsequently, the samples were embedded in paraffin, sectioned into 5  $\mu$ m slices using a Leica RM2135 microtome, and stained with hematoxylin and eosin (H&E). Histopathological examination of the prepared sections was conducted using a Leica light microscope.

### **Statistical analysis**

Data are expressed as mean  $\pm$  standard error of the mean (SEM). Significant differences between groups were assessed using two-way ANOVA followed by Dunnett's post hoc test. All analyses were performed using GraphPad Prism 8 software.

## **Results**

### **Extraction yield**

The yield of alkaloids extracted from the sample was 21.23%. This value represents the percentage of alkaloid compounds obtained relative to the dry weight of the original plant material.

### **Phytochemical screening**

The phytochemical screening revealed the presence of alkaloids, indicated by the formation of a white precipitate.

### **Acute toxicity**

#### **Morphological changes**

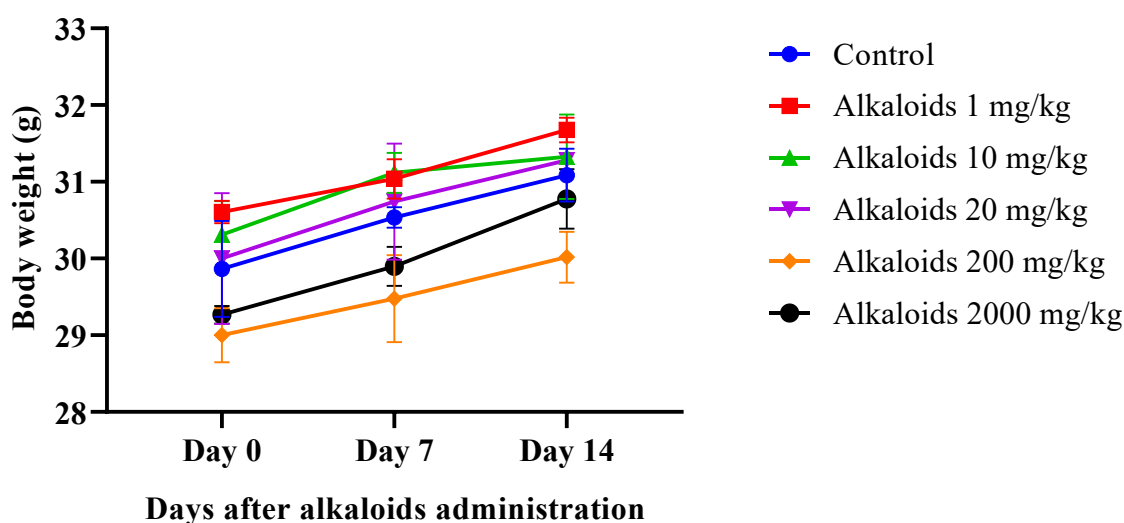
Acute toxicity evaluation revealed no observable toxic effects in either male or female mice at doses of 1, 10, 20, 200, and 2000 mg/kg. No clinical signs of toxicity were detected, including changes in skin or hair, agitation, convulsions, salivation, diarrhea, lethargy, sleep disturbances, coma, eye staining, or respiratory alterations.

#### **Mortality and lethal dose 50**

Evaluation of acute toxicity revealed no mortality in either male or female mice at any of the tested doses (1–2000 mg/kg). Consequently, the lethal dose 50 (LD<sub>50</sub>) value was estimated to be greater than 2000 mg/kg.

#### **Effect of acute toxicity on body weight**

Treatment with alkaloid extracts from *S. olusatrum* resulted in increased body weight in all groups over the 14-day period (**Fig. 1**). Compared to the control group, mice treated at all doses showed weight gains ranging from 1.02 g at 10 and 200 mg/kg to 1.51 g at the highest dose of 2000 mg/kg (**Table 1**). No significant weight loss was observed in any treated group, with the highest dose (2000 mg/kg) producing the most pronounced weight increase (1.51 g) (**Table 1**).



**Fig. 1.** Body weight changes during acute toxicity testing of alkaloids. Body weights of mice were monitored over 14 days. Two-way ANOVA was used to assess significant differences between groups over time. Data are presented as mean  $\pm$  SEM (N = 4 per group).

**Table 1.** Difference in body weight between Day 0 and Day 14 during acute toxicity testing of alkaloids.

| Extracts             | Day 0 (Mean $\pm$ SEM) | Day 14 (Mean $\pm$ SEM) | Difference (g) |
|----------------------|------------------------|-------------------------|----------------|
| Control              | 29.87 $\pm$ 0.62       | 31.09 $\pm$ 0.35        | 1.22           |
| Alkaloids 1 mg/kg    | 30.61 $\pm$ 0.15       | 31.68 $\pm$ 0.16        | 1.07           |
| Alkaloids 10 mg/kg   | 30.31 $\pm$ 0.44       | 31.33 $\pm$ 0.55        | 1.02           |
| Alkaloids 20 mg/kg   | 30 $\pm$ 0.85          | 31.28 $\pm$ 0.03        | 1.28           |
| Alkaloids 200 mg/kg  | 29 $\pm$ 0.35          | 30.02 $\pm$ 0.33        | 1.02           |
| Alkaloids 2000 mg/kg | 29.27 $\pm$ 0.11       | 30.78 $\pm$ 0.39        | 1.51           |

Data are expressed as the mean of 4 mice  $\pm$  SEM.

#### Effect of acute toxicity on relative organ weight

Evaluation of relative organ weights showed no significant differences between control and alkaloid-treated groups at doses ranging from 1 to 2000 mg/kg (**Fig. 2**). The liver exhibited the highest relative weight among all organs; however, its weight variation across the administered doses remained non-significant, ranging from  $1.52 \pm 0.10$  at 1 mg/kg to  $1.83 \pm 0.09$  at 10 mg/kg (**Fig. 2**).

Spleen relative weight ranged from  $0.19 \pm 0.03$  at 1 mg/kg to  $0.22 \pm 0.03$  at 20 mg/kg. Heart relative weight varied from  $0.17 \pm 0.02$  to  $0.20 \pm 0.04$  at 1 and 200 mg/kg, respectively. Kidney weights were between  $0.25 \pm 0.03$  and  $0.30 \pm 0.04$  at 1 mg/kg. Relative weights of lungs, brain, and stomach ranged from 0.36–0.38, 0.38–0.41, and 0.49–0.55, respectively (**Fig. 2**).

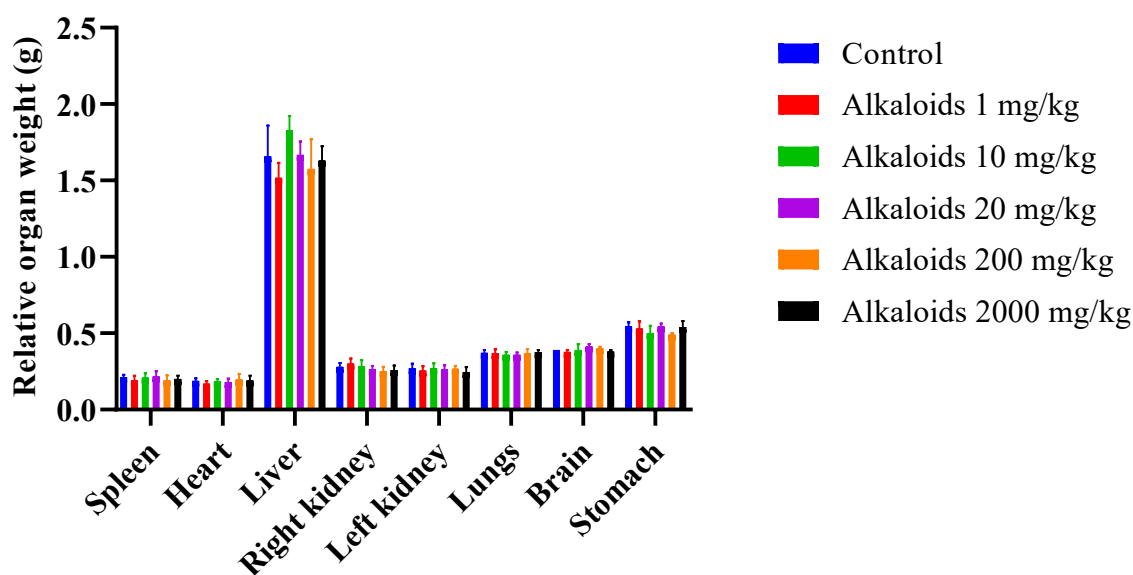


Fig. 2. Relative organ weight in the acute toxicity of alkaloids.

### Subacute toxicity

#### Morphological changes and mortality

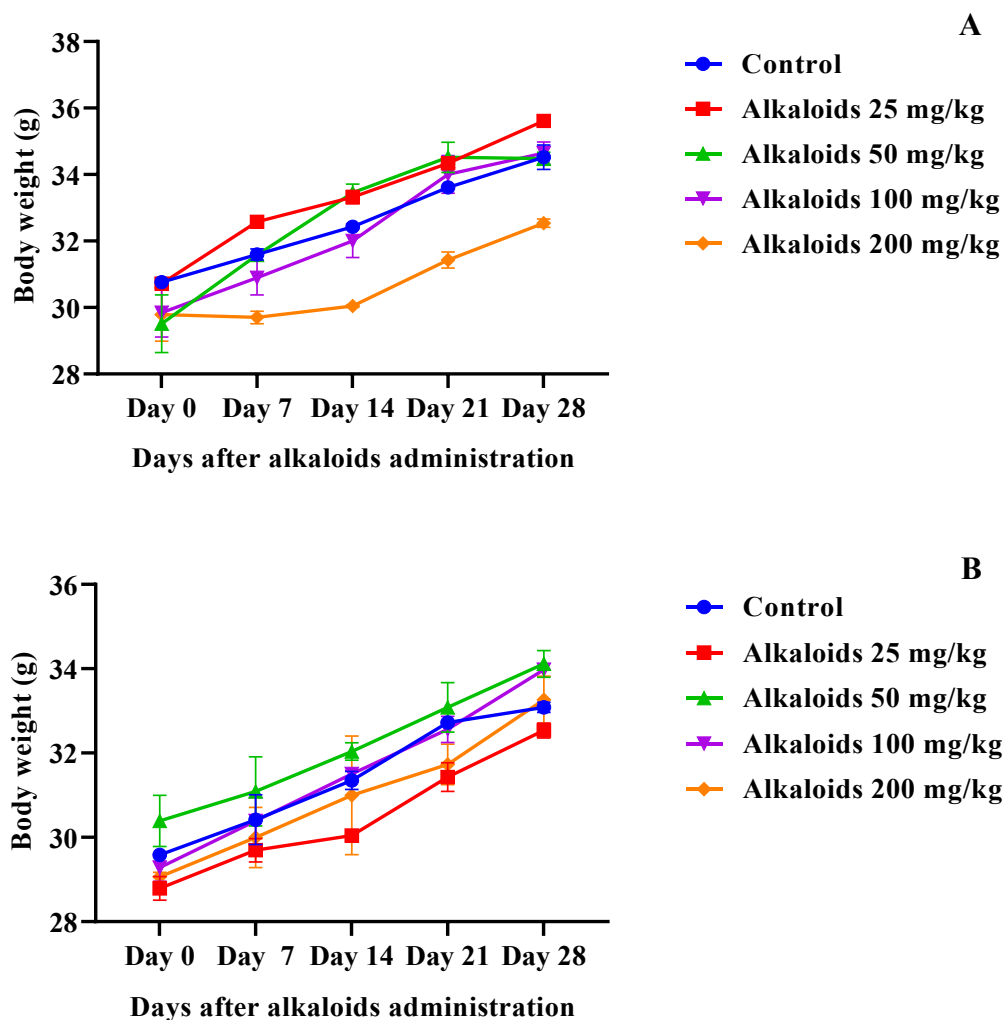
Subacute toxicity assessment over a 28-day period showed that all mice survived at doses of 25, 50, 100, and 200 mg/kg, and no clinical signs of toxicity were observed.

#### Effect of subacute toxicity on body weight

During the 28-day treatment period, both male and female mice in control and alkaloid-treated groups exhibited a steady and progressive increase in body weight (**Fig. 3**).

In male mice, body weight increased in all groups (**Fig. 3A**). Compared to the control group, alkaloid treated males showed similar weight gain patterns, with values ranging from  $30.72 \pm 0.11$  g on day 0 to  $35.61 \pm 0.13$  g on day 28 at 25 mg/kg, and from  $29.79 \pm 0.80$  g to  $32.54 \pm 0.13$  g at 200 mg/kg. No dose-dependent toxicity was observed, as no group showed weight loss or stagnation (**Fig. 3A**).

In female mice, body weight increased steadily over the 28-day period in all groups ( $p < 0.05$ ) (**Fig. 3B**). Control animals gained weight from  $29.58 \pm 0.08$  g on day 0 to  $33.08 \pm 0.08$  g on day 28. Alkaloid-treated groups exhibited similar patterns, with slight variations between doses. Mice treated with 25 mg/kg showed an increase from  $28.79 \pm 0.20$  g to  $32.54 \pm 0.13$  g, while those at 50 mg/kg gained slightly more, from  $30.39 \pm 0.43$  g to  $34.12 \pm 0.23$  g (**Fig. 3B**). At higher doses, weight gain remained consistent, with the 100 mg/kg group increasing from  $29.28 \pm 0.23$  g to  $33.97 \pm 0.01$  g and the 200 mg/kg group from  $29.06 \pm 0.08$  g to  $33.27 \pm 0.39$  g (**Fig. 3B**). Collectively, all treated groups followed the same upward trend as controls, with no evidence of dose-dependent toxicity (**Fig. 3B**).



**Fig. 3.** Effect of subacute alkaloid administration on body weight in mice over 28 days. **(A)** In male mice. **(B)** In female mice.

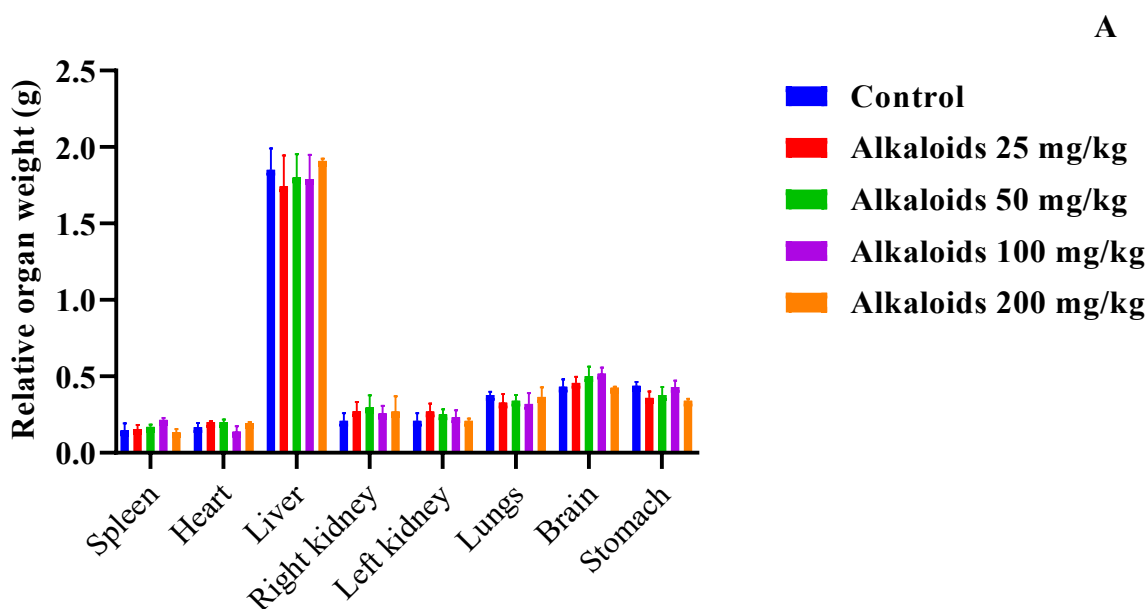
#### Effect of subacute toxicity on relative organ weight

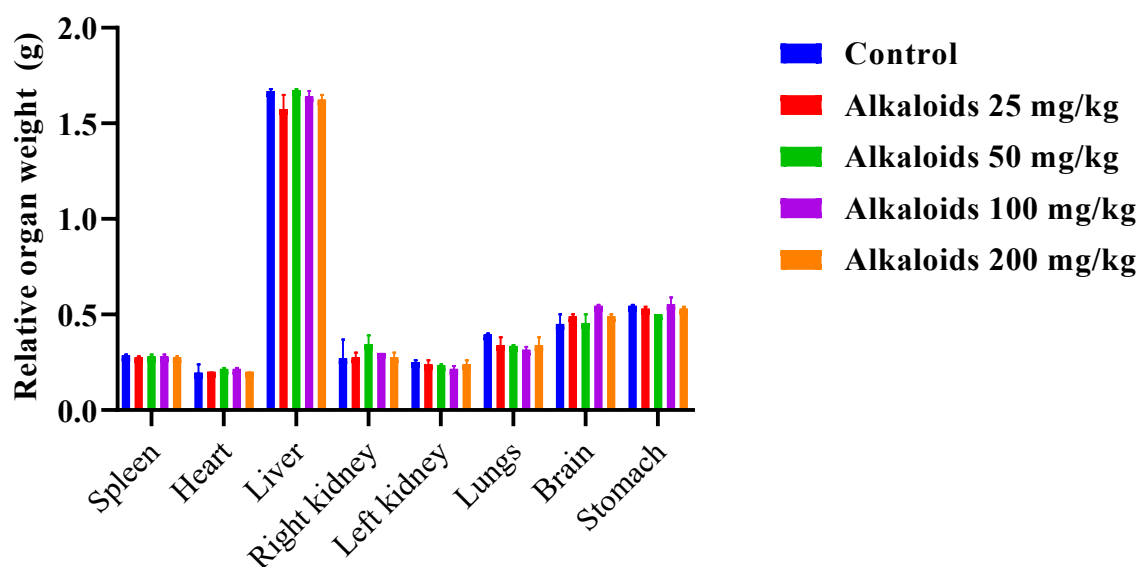
Evaluation of relative organ weights after 28 days of treatment showed no significant differences between control and alkaloid-treated groups at doses of 25, 50, 100, and 200 mg/kg (**Fig. 4**).

In males, the liver exhibited the highest relative weight among all organs; however, its variation across the administered doses remained non-significant, ranging from  $1.74 \pm 0.19$  at 25 mg/kg to  $1.91 \pm 0.01$  at 200 mg/kg (**Fig. 4A**). Spleen relative weight ranged from  $0.14 \pm 0.02$  at 200 mg/kg to  $0.22 \pm 0.01$  at 100 mg/Kg. Heart relative weight varied from  $0.14 \pm 0.03$  at 100 mg/kg to  $0.20 \pm 0.01$  at 200 mg/kg. Kidney weights showed only minor physiological variation, with right kidney values ranging from  $0.27 \pm 0.06$  at 25 mg/kg to  $0.30 \pm 0.08$  at 50 mg/kg, and left kidney values from  $0.21 \pm 0.01$  at 200 mg/kg to  $0.27 \pm 0.05$  at 25 mg/kg, without any dose-dependent trend. Relative weights of lungs,

brain, and stomach remained stable, ranging from 0.32 at 100 mg/kg to 0.36 at 200 mg/kg for lungs, from 0.43 at 200 mg/kg to 0.52 at 100 mg/kg for brain, and from 0.34 at 200 mg/kg to 0.43 at 100 mg/kg for stomach (**Fig. 4A**).

In females, the liver also showed no significant variation, ranging from  $1.58 \pm 0.11$  at 25 mg/kg to  $1.67 \pm 0.01$  at 50 mg/kg (**Fig. 4B**). Spleen relative weight ranged from  $0.275 \pm 0.007$  at 25 mg/kg to  $0.28 \pm 0.01$  at 100 mg/kg. Heart relative weight varied from  $0.20 \pm 0.01$  at 25 mg/kg to  $0.215 \pm 0.007$  at 100 mg/kg. Kidney weights exhibited slight physiological variation, with right kidney values ranging from  $0.28 \pm 0.04$  at 200 mg/kg to  $0.35 \pm 0.06$  at 50 mg/kg, and left kidney values from  $0.215 \pm 0.02$  at 100 mg/kg to  $0.24 \pm 0.03$  at 200 mg/kg, without any dose-dependent trend. Relative weights of lungs, brain, and stomach remained stable, ranging from 0.31 at 100 mg/kg to 0.38 at 200 mg/kg for lungs, from 0.48 at 200 mg/kg to 0.49 at 100 mg/kg for brain, and from  $0.50 \pm 0.01$  at 50 mg/kg to  $0.56 \pm 0.01$  at 100 mg/kg for stomach (**Fig. 4B**).



**B**

**Fig. 4.** Effect of subacute alkaloid administration on relative organ weights in mice after 28 days. **(A)** In male mice. **(B)** In female mice.

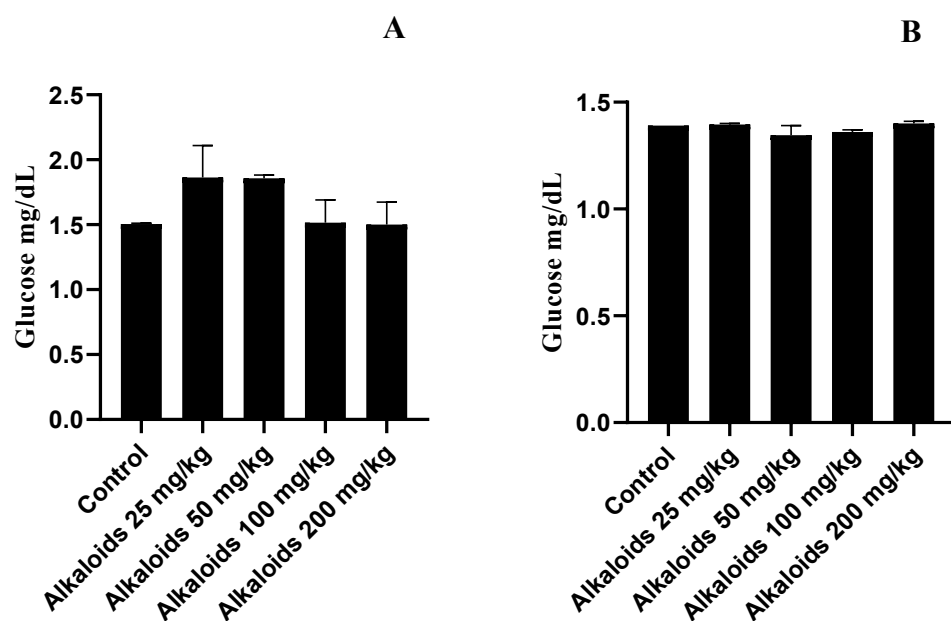
#### Effects of *S. olusatrum* alkaloids on biochemical markers

##### Effect on blood glucose levels

Blood glucose levels were not significantly altered by *S. olusatrum* alkaloid treatment in either male or female mice over the 28-day period (**Fig. 5**).

In males, glucose concentrations ranged from  $1.86 \pm 0.23$  mg/dL at 25 mg/kg to  $1.86 \pm 0.02$  mg/dL,  $1.52 \pm 0.19$  mg/dL, and  $1.50 \pm 0.17$  mg/dL at 50, 100, and 200 mg/kg, respectively (**Fig. 5A**). Values recorded at 100 and 200 mg/kg were comparable to those of the control group ( $1.51 \pm 0.01$  mg/dL).

In females, glucose levels remained stable across all treated groups, ranging from  $1.35 \pm 0.004$  mg/dL at 50 mg/kg to  $1.40 \pm 0.01$  mg/dL at 200 mg/kg. No significant differences were observed compared to the control group (**Fig. 5B**).



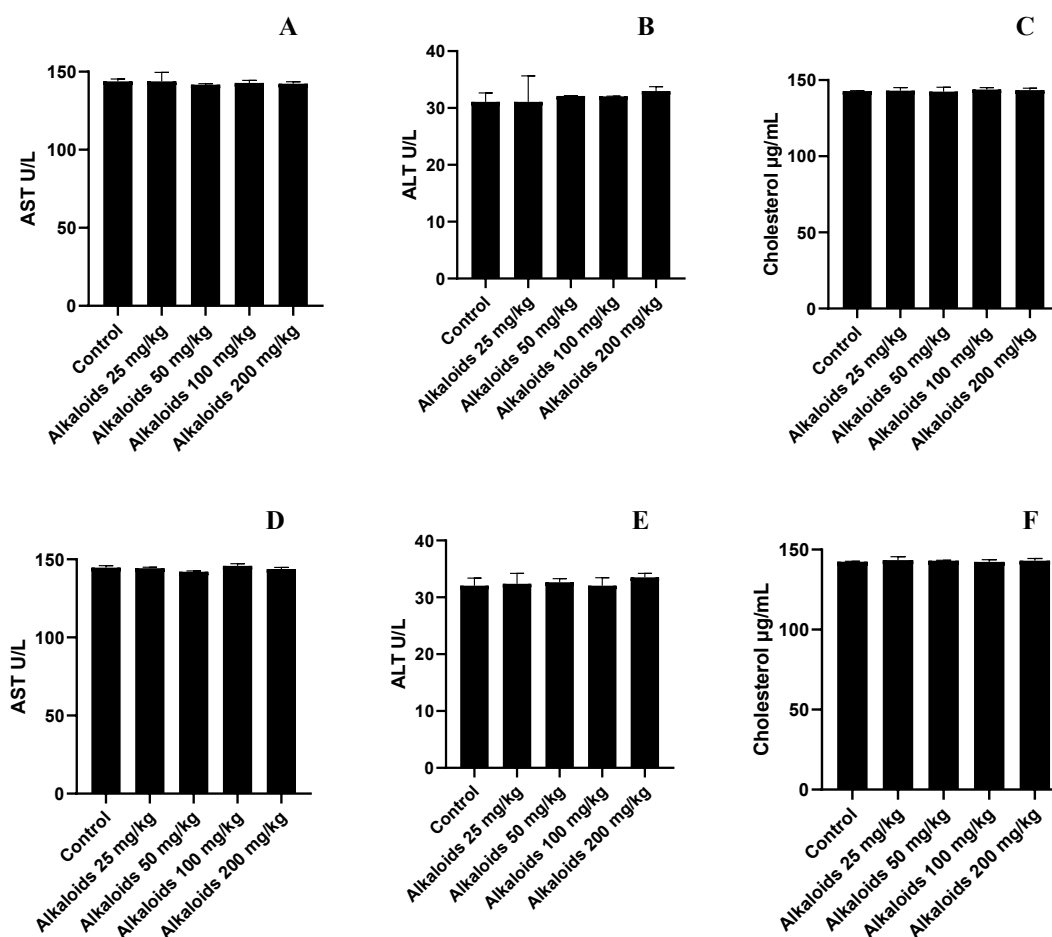
**Fig. 5.** Effect of *S. olusatrum* alkaloids on blood glucose levels in mice. **(A)** In male mice. **(B)** In female mice.

#### Effect on liver function parameters

Assessment of liver enzymes and cholesterol showed that *S. olusatrum* alkaloid treatment did not induce significant alterations in male or female mice over 28 days (**Fig. 6**).

Compared to control, AST levels remained stable across all treated groups, ranging from  $143.79 \pm 0.01$  U/L at 25 mg/kg to  $142 \pm 0.71$  U/L at 200 mg/kg, with intermediate values of  $141.71 \pm 0.02$  U/L at 50 mg/kg and  $142.77 \pm 1.06$  U/L at 100 mg/kg in males (**Fig. 6A**). In males, ALT activity also showed no significant variation compared to control, ranging from  $31.09 \pm 0.01$  U/L at 25 mg/kg to  $33 \pm 1$  U/L at 200 mg/kg, with intermediate values of  $32.10 \pm 0.01$  U/L at 50 mg/kg and  $32.04 \pm 0.04$  U/L at 100 mg/kg (**Fig. 6B**). Cholesterol concentrations remained stable compared to control, ranging from  $141.93 \pm 2.78$   $\mu$ g/mL at 25 mg/kg to  $145.60 \pm 0.01$   $\mu$ g/mL at 200 mg/kg, with intermediate values of  $137.27 \pm 7.14$   $\mu$ g/mL at 50 mg/kg (**Fig. 6C**).

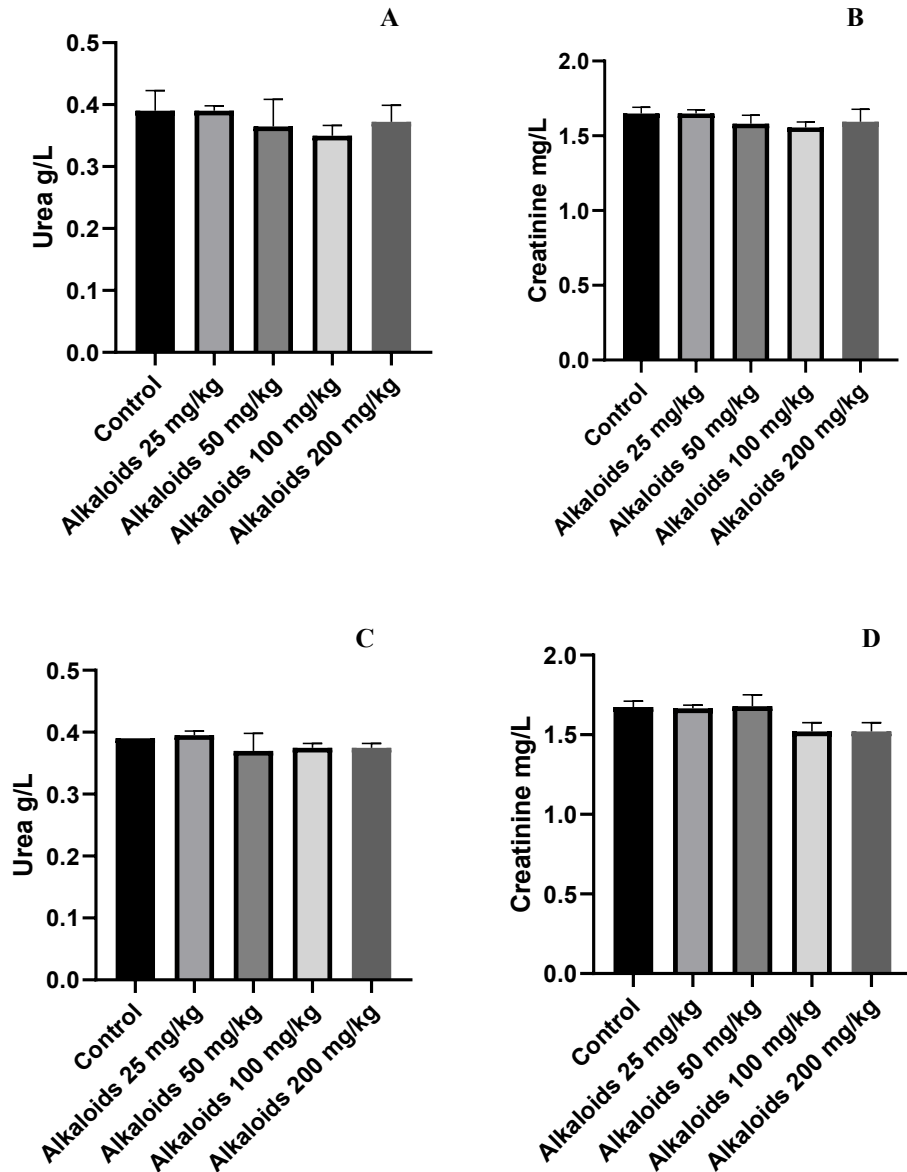
Compared to control, in females, AST levels showed minimal variation across all treated groups, ranging from  $143.79 \pm 2.01$  U/L at 25 mg/kg to  $144.50 \pm 0.71$  U/L at 200 mg/kg, with intermediate values of  $141.71 \pm 0.53$  U/L at 50 mg/kg and  $145.82 \pm 0.95$  U/L at 100 mg/kg (**Fig. 6D**). ALT activity remained unchanged compared to control, ranging from  $31.09 \pm 5$  U/L at 25 mg/kg to  $34 \pm 1.90$  U/L at 200 mg/kg, with intermediate values of  $33.10 \pm 0.14$  U/L at 50 mg/kg and  $33.04 \pm 1.35$  U/L at 100 mg/kg (**Fig. 6E**). Similarly, cholesterol levels were comparable to control, ranging from  $141.93 \pm 1.41$   $\mu$ g/mL at 25 mg/kg to  $144 \pm 0.99$   $\mu$ g/mL at 200 mg/kg, with intermediate values of  $142.93 \pm 2.03$   $\mu$ g/mL at 50 mg/kg and  $143.27 \pm 0.80$   $\mu$ g/mL at 100 mg/kg (**Fig. 6F**).



**Fig. 6.** Effect of *S. olusatrum* alkaloids on liver function and cholesterol levels in mice. **(A)** AST in male mice. **(B)** ALT in male mice. **(C)** Cholesterol in male mice. **(D)** AST in female mice. **(E)** ALT in female mice. **(F)** Cholesterol in female mice.

### Effect on kidney function parameters

Analysis of renal biomarkers showed stable urea and creatinine levels in both sexes throughout the experimental period when compared with the control group (**Fig. 7**). No dose or sex-dependent variations were detected. In male mice, urea concentrations remained comparable to control values ( $1.66 \pm 0.02$  g/L), ranging from  $1.55 \pm 0.05$  g/L to  $1.66 \pm 0.02$  g/L across all tested doses, while creatinine levels showed no deviation from the control ( $0.39 \pm 0.01$  mg/L), varying between  $0.37 \pm 0.01$  mg/L and  $0.39 \pm 0.01$  mg/L (**Fig. 7A, B**). A similar profile was observed in females, with urea values remaining close to control levels ( $0.39 \pm 0.01$  g/L) and ranging from  $0.37 \pm 0.01$  g/L to  $0.39 \pm 0.01$  g/L, and creatinine levels comparable to the control ( $1.69 \pm 0.03$  mg/L), ranging from  $1.48 \pm 0.01$  g/L to  $1.69 \pm 0.03$  g/L (**Fig. 7C, D**).



**Fig. 7.** Effect of *S. olusatrum* alkaloids on renal function biomarkers in mice. (A) Urea levels in male mice. (B) Creatinine levels in male mice. (C) Urea levels in female mice. (D) Creatinine levels in female mice.

#### Effect of *S. olusatrum* alkaloids on hematological parameters

##### Effect on red blood cells

Subacute intraperitoneal administration of alkaloids did not induce significant alterations in hematological parameters in male or female mice (Table 2).

In males, compared to control, RBC counts ranged from  $8.36 \pm 0.27 \times 10^6/\mu\text{L}$  at 25 mg/kg to  $7.63 \pm 0.08 \times 10^6/\mu\text{L}$  at 50 mg/kg,  $7.96 \pm 0.54 \times 10^6/\mu\text{L}$  at 100 mg/kg, and  $7.70 \pm 0.60 \times 10^6/\mu\text{L}$  at 200 mg/kg. HB levels varied from  $12.80 \pm 0.05$  g/dL at 25 mg/kg to  $12.66 \pm 0.08$  g/dL at 50 mg/kg,  $12.06 \pm 0.21$  g/dL at 100 mg/kg, and  $12.80 \pm 0.44$  g/dL at 200 mg/kg, compared to control ( $12.36 \pm 0.14$  g/dL). HT values showed slight variations compared to control ( $36.36$

$\pm 0.53\%$ ), ranging from  $37.50 \pm 0.30\%$  at 25 mg/kg to  $34.06 \pm 1.58\%$  at 50 mg/kg,  $35.26 \pm 0.20\%$  at 100 mg/kg, and  $35.20 \pm 0.22\%$  at 200 mg/kg. Additionally, MCV, MCH, MCHC, and RDW remained comparable to control across all doses. MCV ranged from  $55 \pm 0.57$  fL at 25 mg/kg to  $54 \pm 2.08$  fL at 50 mg/kg,  $55.33 \pm 0.88$  fL at 100 mg/kg, and  $55.30 \pm 0.88$  fL at 200 mg/kg (control:  $53 \pm 0.57$  fL). RDW varied from  $24.03 \pm 0.08\%$  at 25 mg/kg to  $23.23 \pm 0.64\%$  at 50 mg/kg,  $23.96 \pm 2.05\%$  at 100 mg/kg, and  $23.70 \pm 1.85\%$  at 200 mg/kg (control:  $23.53 \pm 1.91\%$ ) (**Table 2**).

In females, compared to control, RBC counts ranged from  $8.26 \pm 0.27 \times 10^6/\mu\text{L}$  at 25 mg/kg to  $7.50 \pm 0.17 \times 10^6/\mu\text{L}$  at 50 mg/kg,  $7.76 \pm 0.43 \times 10^6/\mu\text{L}$  at 100 mg/kg, and  $8.03 \pm 0.81 \times 10^6/\mu\text{L}$  at 200 mg/kg. HB levels varied from  $11.63 \pm 0.17$  g/dL at 25 mg/kg to  $11.80 \pm 0.05$  g/dL at 50 mg/kg,  $12.96 \pm 0.29$  g/dL at 100 mg/kg, and  $12.13 \pm 0.18$  g/dL at 200 mg/kg, compared to control ( $12.23 \pm 0.09$  g/dL). HT values ranged from  $37.23 \pm 0.28\%$  at 25 mg/kg to  $36.43 \pm 0.34\%$  at 50 mg/kg,  $35.06 \pm 0.34\%$  at 100 mg/kg, and  $35.50 \pm 0.53\%$  at 200 mg/kg (control:  $36.10 \pm 0.49\%$ ). MCV, MCH, MCHC, and RDW showed no marked dose-dependent changes compared to control. MCV ranged from  $58.66 \pm 0.33$  fL at 25 mg/kg to  $59 \pm 1.15$  fL at 50 mg/kg,  $57.66 \pm 1.33$  fL at 100 mg/kg, and  $55.67 \pm 0.67$  fL at 200 mg/kg (control:  $57 \pm 1.52$  fL). RDW varied from  $23.96 \pm 0.03\%$  at 25 mg/kg to  $23.06 \pm 0.63\%$  at 50 mg/kg,  $23.33 \pm 1.20\%$  at 100 mg/kg, and  $24 \pm 1.53\%$  at 200 mg/kg (control:  $24.33 \pm 0.33\%$ ) (**Table 2**).

**Table 2.** Effect of subacute alkaloid administration on hematological parameters in male and female mice.

| Sex/Dose (mg/kg) | RBC (×10 <sup>6</sup> /μL) | HB (g/dL)   | HT (%)       | MCV (fL)     | MCH (pg)     | MCHC (g/dL)  | RDW (%)      |              |
|------------------|----------------------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Male             | Control                    | 7.4 ± 0.61  | 12.36 ± 0.14 | 36.36 ± 0.53 | 53 ± 0.57    | 14.93 ± 0.08 | 28.43 ± 0.33 | 23.53 ± 1.91 |
|                  | 25 mg/kg                   | 8.36 ± 0.27 | 12.8 ± 0.05  | 37.5 ± 0.3   | 55 ± 0.57    | 15.3 ± 0.15  | 28.33 ± 0.33 | 24.03 ± 0.08 |
|                  | 50 mg/kg                   | 7.63 ± 0.08 | 12.66 ± 0.08 | 34.06 ± 1.58 | 54 ± 2.08    | 14.16 ± 0.08 | 28.5 ± 0.28  | 23.23 ± 0.64 |
|                  | 100 mg/kg                  | 7.96 ± 0.54 | 12.06 ± 0.21 | 35.26 ± 0.2  | 55.33 ± 0.88 | 16.23 ± 0.12 | 28.5 ± 0.28  | 23.96 ± 2.05 |
|                  | 200 mg/kg                  | 7.7 ± 0.6   | 12.8 ± 0.44  | 35.2 ± 0.22  | 55.3 ± 0.88  | 16.1 ± 0.1   | 28.3 ± 0.33  | 23.7 ± 1.85  |
| Female           | Control                    | 7.1 ± 0.58  | 12.23 ± 0.09 | 36.1 ± 0.49  | 57 ± 1.52    | 15.63 ± 0.58 | 28.36 ± 0.36 | 24.33 ± 0.33 |
|                  | 25 mg/kg                   | 8.26 ± 0.27 | 11.63 ± 0.17 | 37.23 ± 0.28 | 58.66 ± 0.33 | 16.13 ± 0.13 | 28.66 ± 0.33 | 23.96 ± 0.03 |
|                  | 50 mg/kg                   | 7.5 ± 0.17  | 11.8 ± 0.05  | 36.43 ± 0.34 | 59 ± 1.15    | 15.1 ± 0.49  | 28.33 ± 0.33 | 23.06 ± 0.63 |
|                  | 100 mg/kg                  | 7.76 ± 0.43 | 12.96 ± 0.29 | 35.06 ± 0.34 | 57.66 ± 1.33 | 16.1 ± 0.1   | 28.33 ± 0.33 | 23.33 ± 1.2  |
|                  | 200 mg/kg                  | 8.03 ± 0.81 | 12.13 ± 0.18 | 35.50 ± 0.53 | 55.67 ± 0.67 | 16.43 ± 0.21 | 28.67 ± 0.33 | 24 ± 1.53    |

Data are presented as mean  $\pm$  SEM. No significant alterations in hematological parameters were observed.

### Effect on platelet and white blood cells count

Evaluation of the white blood cell profile revealed that platelet counts and leukocyte composition were not significantly affected by *S. olusatrum* alkaloid treatment in male or female mice (**Table 3**).

In males, compared to control, platelet counts remained stable across all treated groups, ranging from  $1231 \pm 15.04 \times 10^3/\mu\text{L}$  at 25 mg/kg to  $1217 \pm 16.50 \times 10^3/\mu\text{L}$  at 100 mg/kg,  $1232 \pm 15.66 \times 10^3/\mu\text{L}$  at 50 mg/kg, and  $1227 \pm 5.20 \times 10^3/\mu\text{L}$  at 200 mg/kg. Neutrophil percentages showed slight variation across doses, ranging from  $24 \pm 1.52\%$  at 25 mg/kg to  $28 \pm 0.58\%$  at 200 mg/kg, with intermediate values of  $24.33 \pm 0.33\%$  at 50 mg/kg and  $25.66 \pm 0.33\%$  at 100 mg/kg. Lymphocyte percentages remained stable compared to control, ranging from  $76 \pm 1.52\%$  at 25 mg/kg to  $72 \pm$

0.58% at 200 mg/kg, with intermediate values of  $75.67 \pm 0.33\%$  at 50 mg/kg and  $74.34 \pm 0.33\%$  at 100 mg/kg. Eosinophils, basophils, and monocytes were not detected in any treated or control group (**Table 3**).

In females, compared to control, platelet counts remained stable across all doses, ranging from  $1237 \pm 0.57 \times 10^3/\mu\text{L}$  at 25 mg/kg to  $1229 \pm 6.50 \times 10^3/\mu\text{L}$  at 200 mg/kg, with intermediate values of  $1231 \pm 15.43 \times 10^3/\mu\text{L}$  at 50 mg/kg and  $1238 \pm 19.61 \times 10^3/\mu\text{L}$  at 100 mg/kg. Neutrophil percentages varied slightly across doses, ranging from  $27.66 \pm 1.85\%$  at 25 mg/kg to  $29.66 \pm 1.20\%$  at 50 mg/kg,  $29 \pm 0.57\%$  at 100 mg/kg, and  $28.70 \pm 0.88\%$  at 200 mg/kg. Lymphocyte percentages remained comparable to control, ranging from  $72.34 \pm 1.85\%$  at 25 mg/kg to  $70.34 \pm 1.20\%$  at 50 mg/kg,  $71 \pm 0.57\%$  at 100 mg/kg, and  $71.30 \pm 0.88\%$  at 200 mg/kg. Eosinophils, basophils, and monocytes were not detected in any group (**Table 3**).

**Table 3.** Effect of *S. olusatrum* alkaloids on platelet and white blood cell counts in male and female mice.

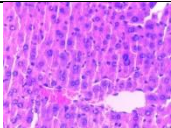
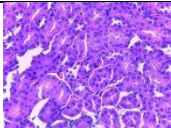
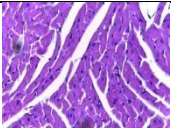
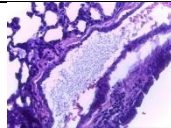
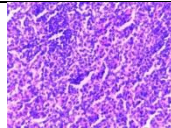
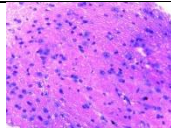
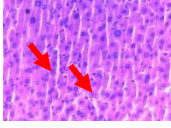
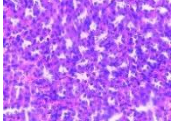
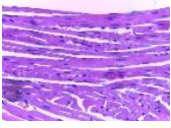
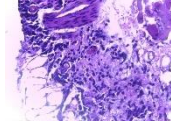
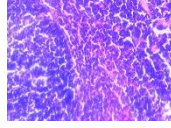
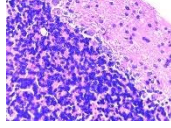
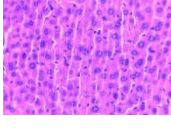
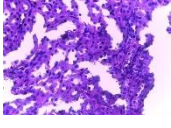
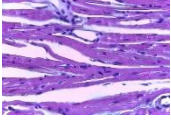
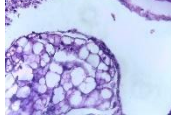
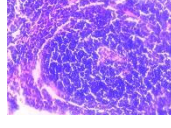
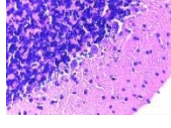
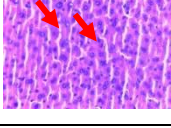
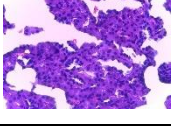
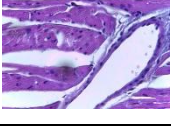
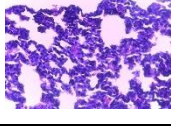
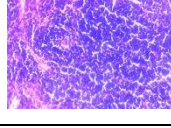
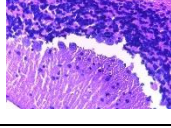
| Sex/Dose (mg/Kg) | Platelets ( $\times 10^3/\mu\text{L}$ ) | Neutrophils (%)  | Eosinophils (%) | Basophils (%) | Lymphocytes (%)  | Monocytes (%) |
|------------------|-----------------------------------------|------------------|-----------------|---------------|------------------|---------------|
| <b>Male</b>      |                                         |                  |                 |               |                  |               |
| Control          | $1216 \pm 15.83$                        | $24.33 \pm 0.33$ | 0               | 0             | $75.67 \pm 0.33$ | 0             |
| 25 mg/kg         | $1231 \pm 15.04$                        | $24 \pm 1.52$    | 0               | 0             | $76 \pm 1.52$    | 0             |
| 50 mg/kg         | $1232 \pm 15.66$                        | $24.33 \pm 0.33$ | 0               | 0             | $75.67 \pm 0.33$ | 0             |
| 100 mg/kg        | $1217 \pm 16.50$                        | $25.66 \pm 0.33$ | 0               | 0             | $74.34 \pm 0.33$ | 0             |
| 200 mg/kg        | $1227 \pm 5.2$                          | $28 \pm 0.58$    | 0               | 0             | $72 \pm 0.58$    | 0             |
| <b>Female</b>    |                                         |                  |                 |               |                  |               |
| Control          | $1233 \pm 16.01$                        | $29.33 \pm 0.88$ | 0               | 0             | $70.67 \pm 0.88$ | 0             |
| 25 mg/kg         | $1237 \pm 0.57$                         | $27.66 \pm 1.85$ | 0               | 0             | $72.34 \pm 1.85$ | 0             |
| 50 mg/kg         | $1231 \pm 15.43$                        | $29.66 \pm 1.20$ | 0               | 0             | $70.34 \pm 1.20$ | 0             |
| 100 mg/kg        | $1238 \pm 19.61$                        | $29 \pm 0.57$    | 0               | 0             | $71 \pm 0.57$    | 0             |
| 200 mg/kg        | $1229 \pm 6.5$                          | $28.70 \pm 0.88$ | 0               | 0             | $71.30 \pm 0.88$ | 0             |

Data are presented as mean  $\pm$  SEM. No significant alterations in leukocyte composition were detected.

### 1.1. Histological analysis of vital organs

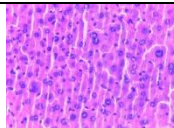
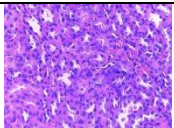
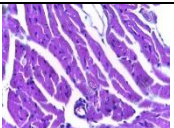
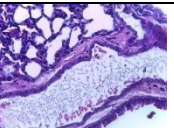
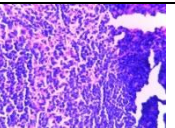
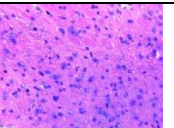
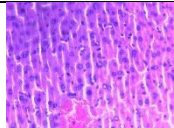
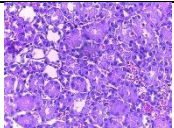
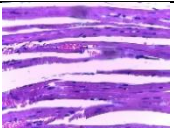
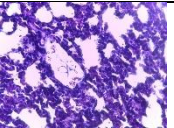
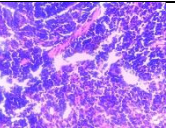
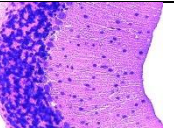
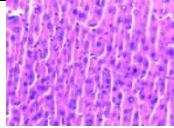
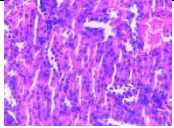
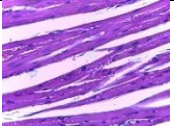
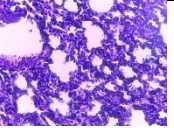
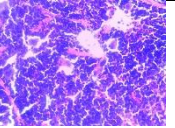
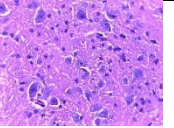
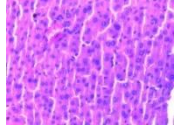
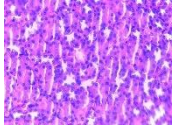
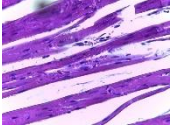
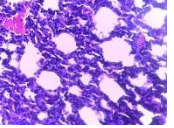
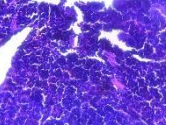
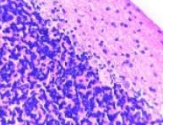
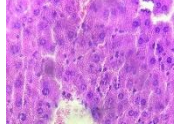
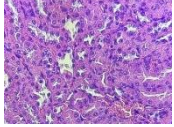
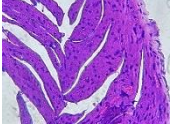
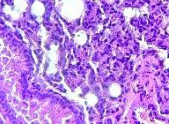
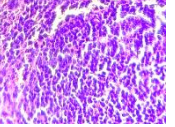
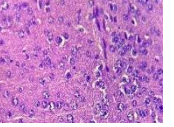
A detailed histopathological analysis across all examined organs revealed no significant morphological abnormalities in any of the treated groups compared to controls in both males and females, with the exception of the liver, which showed sex-dependent changes (**Tables 4, 5**). In male mice, mild disorganization of hepatocyte cords and slight widening of sinusoidal spaces were observed at intermediate doses (25 and 100 mg/kg), indicating subtle alterations in hepatocellular cohesion (**Table 4**). Female mice exhibited well-preserved hepatic architecture at all doses, with regularly arranged hepatocyte cords, intact sinusoidal spaces, and no evidence of cellular disorganization or inflammatory infiltration (**Table 5**). Renal tissues displayed normal architecture with well-delineated cortical and medullary regions. Glomeruli were evenly distributed and surrounded by thin Bowman's capsules, while the tubular system, including proximal and distal convoluted tubules, Henle's loops, and collecting ducts, remained structurally intact without signs of inflammation, degeneration, or fibrosis (**Tables 4, 5**). Cardiac sections revealed orderly myocardial fibers with distinct transverse striations and centrally located nuclei. The endocardium and epicardium appeared unremarkable, with no evidence of necrosis, inflammatory infiltration, or vascular damage (**Tables 4, 5**). Pulmonary tissue showed well-preserved alveolar structures, and bronchioles and vascular components maintained normal morphology across all dose groups (**Tables 4, 5**). The spleen retained its characteristic histological architecture with intact white and red pulp regions, well-defined lymphoid follicles, and periarteriolar sheaths, with no signs of hyperplasia, congestion, or architectural disorganization (**Tables 4, 5**).

**Table 5.** Histopathological evaluation of major organs following subacute intraperitoneal administration of *S. olusatrum* alkaloids in male mice.

|                     | Liver                                                                             | Kidneys                                                                           | Heart                                                                             | Lungs                                                                              | Spleen                                                                              | Brain                                                                               |
|---------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Control             |  |  |  |  |  |  |
| Alkaloids 25 mg/kg  |  |  |  |  |  |  |
| Alkaloids 50 mg/kg  |  |  |  |  |  |  |
| Alkaloids 100 mg/kg |  |  |  |  |  |  |

Sections were stained with hematoxylin and eosin and examined under light microscopy at  $\times 40$  magnification. Histopathological evaluation revealed no organ-specific damage, except for mild alterations in the liver

**Table 6.** Histopathological evaluation of major organs following subacute intraperitoneal administration of *S. olusatrum* alkaloids in female mice.

|                     | Liver                                                                              | Kidneys                                                                            | Heart                                                                              | Lungs                                                                               | Spleen                                                                               | Brain                                                                                |
|---------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Control             |   |   |   |   |   |   |
| Alkaloids 25 mg/kg  |   |   |   |   |   |   |
| Alkaloids 50 mg/kg  |   |   |   |   |   |   |
| Alkaloids 100 mg/kg |   |   |   |   |   |   |
| Alkaloids 200 mg/kg |  |  |  |  |  |  |

Sections were stained with hematoxylin and eosin and examined under light microscopy at  $\times 40$  magnification. No histopathological alterations were observed in any organ across all doses.

## Discussion

Toxicological evaluation of medicinal and aromatic plant extracts and their bioactive constituents is essential for defining toxic and subtoxic dose ranges, safety margins, and their potential for pharmacological exploitation. In this regard, the limited toxicological data available on *S. olusatrum* prompted the present study, which aimed to investigate the acute and subacute toxicity of its alkaloid fraction and to better delineate its safety profile.

Phytochemical analysis indicated that *S. olusatrum* is a relatively rich source of alkaloids, an observation not previously well established. This finding was further supported by phytochemical screening, confirming the presence of alkaloids and validating the extraction approach employed.

Acute toxicity assessment revealed no observable signs of toxicity across the tested dose range. The absence of significant alterations in body weight and relative organ weights further supports a low toxicity profile in mice. These findings are consistent with reports on other alkaloid-containing plant extracts, such as *Dysphania ambrosioides*, which exhibits an oral LD<sub>50</sub> of approximately 5000 mg/kg (Kandsi et al. 2022). In contrast, highly toxic alkaloids, such as piperidine derivatives from *Conium maculatum*, induce lethality at doses exceeding 20 mg/kg (Radulović et al. 2012; Al-Snafi 2016), highlighting the structure-dependent nature of alkaloid toxicity.

The LD<sub>50</sub> value of *S. olusatrum* alkaloids (>2000 mg/kg) indicates good tolerability and places the extract within the low-toxicity category according to international classification systems (Mugale et al., 2024). This suggests a relatively wide therapeutic aspect, which is a critical prerequisite for drug development. These findings are consistent with previous reports from our group (El Hamsas el Youbi et al., 2024), where ethanolic and methanolic extracts exhibited LD<sub>50</sub> values exceeding 5000 mg/kg, thereby reinforcing the reliability of the observed safety profile across different extraction systems.

To further contextualize these results, it is important to consider the toxicological variability among other secondary metabolites of *S. olusatrum*. Isofuranodiene, a major constituent, has been reported to exhibit low toxicity in biological models, with mortality rates below 7% in *Culex quinquefasciatus* at 1000 mg/kg after 14 days of exposure (Pavela et al. 2019). However, its relatively low LD<sub>50</sub> values (15.8 µg/cm<sup>2</sup> acute; 11.9 µg/cm<sup>3</sup> chronic) suggest that toxicity may vary substantially depending on exposure conditions and biological systems. By contrast, germacrene demonstrated higher LD<sub>50</sub> values (>100 µg/cm<sup>2</sup> acute; 42.7 µg/cm<sup>3</sup> chronic), indicating comparatively lower toxicity (Benelli et al. 2017).

Regarding subacute toxicity, repeated intraperitoneal administration of *S. olusatrum* alkaloids did not induce any significant changes in body weight or relative organ weights in either male or female mice. These findings indicate good systemic tolerance and suggest that the extract does not interfere with overall metabolic homeostasis at the tested doses. These observations are in agreement with previous studies on alkaloid-containing plant extracts. For instance, *Dysphania ambrosioides* alkaloids have been shown not to significantly affect food intake, water consumption, body weight, or organ weights in subacute toxicity models (Kandsi et al. 2022). However, certain alkaloid classes, such as ergot alkaloids, are well known to induce marked metabolic disturbances including anorexia, weight loss, and multi-

organ dysfunction, emphasizing again the importance of structural diversity in determining toxicological outcomes (Agriopoulou 2021; Akinboye et al. 2023).

Biochemical, hematological, and histopathological evaluations are considered essential components of toxicity assessment, as they provide sensitive indicators of systemic and organ-specific effects (Debelo et al. 2016). In the present study, no significant alterations were observed in serum biochemical parameters, including AST, ALT, urea, creatinine, glucose, and cholesterol levels. These findings suggest that the alkaloid fraction of *S. olusatrum* does not impair hepatic, renal, or endocrine functions, nor does it disrupt lipid or carbohydrate metabolism. Similarly, hematological analysis revealed no significant changes in erythrocyte, leukocyte, or platelet parameters in either sex. These results indicate the absence of hematotoxic effects such as anemia, leukopenia, neutropenia, or thrombocytopenia, which are often early indicators of systemic toxicity (Lubran 1989; Arika et al. 2016).

Histopathological examination further supported these findings, revealing no major structural alterations in vital organs following repeated administration. The overall integrity of hepatic, renal, and other examined tissues confirm the absence of overt organ toxicity. These results are consistent with previous reports on plant-derived alkaloid extracts, which generally exhibit minimal histological alterations under subacute exposure conditions (Brígido et al. 2021). An exception was observed in the liver of male mice, where mild, dose-dependent histoarchitectural changes were noted, including slight disorganization of hepatocyte cords and moderate sinusoidal dilation. Importantly, these changes were not associated with inflammation, necrosis, or cellular degeneration, suggesting adaptive rather than pathological responses. Such mild hepatic alterations are frequently interpreted as reversible adaptive responses to xenobiotic exposure, particularly in organs involved in detoxification and biotransformation (Williams and Iatropoulos 2002; Hall et al. 2012). The liver, as the primary site of xenobiotic metabolism, may exhibit transient structural remodeling without causing necrosis or inflammation (Williams and Iatropoulos 2002). The sex-dependent nature of these effects may be related to differences in hormonal regulation between male and female mice (Waxman and Holloway 2009; Hochmuth et al. 2021).

## Conclusion

The present study demonstrates that alkaloid-rich extracts from *S. olusatrum* seeds exhibit low acute and subacute toxicity in Swiss mice. No mortality, behavioral abnormalities, or significant changes in body weight, relative organ weights, hematological profiles, or biochemical markers were observed across the tested doses. Histopathological analyses further confirmed the absence of major tissue damage in vital organs, with the exception of mild, dose-dependent, and sex-dependent changes in the liver of male mice, likely reflecting adaptive hepatocellular responses to xenobiotic metabolism rather than overt toxicity. These findings indicate that *S. olusatrum* seed alkaloids are generally well-tolerated, supporting their potential safe use in pharmacological applications, while highlighting the importance of dose optimization and consideration of sex-related differences in hepatic responses. Collectively, this study provides a comprehensive toxicological profile that can serve as a reference for the safe development of *S. olusatrum*-derived bioactive compounds.

**Abbreviations**

**ALT** : Alanine aminotransferase

**AST** : Aspartate aminotransferase

**HB** : Hemoglobin

**LD<sub>50</sub>** : Lethal dose 50

**MCH** : Mean corpuscular hemoglobin

**MCHC** : Mean corpuscular hemoglobin concentration

**MCV** : Mean corpuscular volume

**RBC** : Red blood cells

**RDW** : Red cell distribution width

**WBC** : White blood cells

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## **Statements and Declarations**

### **Competing Interests**

The authors declare that they have no known competing financial or non-financial interests that could have appeared to influence the work reported in this paper.

### **Conflict of interest**

The authors declare that they have no conflict of interests.

### **Data availability**

No datasets were generated or analysed during the current study.

### **Author contributions**

Z.S.: Writing—original draft preparation, methodology, formal analysis, investigation, visualization, and data curation. O.B.: Investigation and software. D.R.: Writing—review and editing. N.E.A.: Writing—review and editing. E.E.A.: Supervision and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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### **Ethics declarations**

The animal study protocol was approved by the Institutional Ethics Committee of the Faculty of Sciences Ben M'Sik, Hassan II University of Casablanca, Morocco (protocol code: CEEA/2025/001). All experimental procedures were conducted in accordance with internationally recognized guidelines for the care and use of laboratory animals.

### **Declaration of generative AI use**

Generative artificial intelligence tools, including Grammarly and ChatGPT (online versions), were used exclusively to support linguistic revision, improve clarity, and enhance the academic writing style of the manuscript. All scientific concepts, data analyses, interpretations, and conclusions were independently developed, critically evaluated, and validated by the authors.

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The authors used a large language model (ChatGPT, OpenAI) solely for language editing and improving readability of the manuscript. The scientific content, experimental design, data analysis, and interpretation of results were entirely performed by the authors, who take full responsibility for the final version of the manuscript.