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# ACUTE TOXICITY STUDY OF SESQUITERPENE LACTONES-ENRICHED FRACTION OF *CICHORIUM INTYBUS* IN RATS

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## ABSTRACT

*Cichorium intybus* has a wide range of therapeutic uses. This study aimed to investigate the acute toxic effect of the sesquiterpene lactone-enriched fraction of *C. intybus* in Wistar rats, following the Organization for Economic Cooperation and Development (OECD) Guideline 423. Thirty healthy female rats were divided equally into three groups. The first one was control. The second and third groups received a single dose of chicory fraction 2000 and 3000 mg/kg b.wt., respectively. In the 1st week after dosing, results showed a marked reduction in weight gain in both treated groups, with the death of 50% of the rats receiving 3000 mg/kg b.wt. Behavior test results revealed that chicory fraction at 2000 mg/kg b.wt. had a distinct anxiolytic effect and increased locomotor activity, while the highest dose of chicory fraction had anxiogenic action. Statistically significant leucopenia accompanied by absolute lymphopenia, with reduction in serum hepatic transaminase at both doses, has been noticed. Besides the haemato-biochemical alterations induced by chicory fraction, the hepatic tissues showed congestion and leucocytic infiltration, with eosinophilic reaction, especially with the highest dose. The renal tissue exhibited congestion and features of nephrosis in the form of vacuolation and hyaline droplets. In conclusion, the maximum safety dose of *Cichorium intybus* sesquiterpene lactones-enriched fraction is 2000 mg/kg b.wt. as it has an anxiolytic effect. While the higher dose (3000 mg/kg b.wt.) has an anxiogenic effect, 50% mortality of rats, indicating a marked toxicological effect.

***Key words**: Cichorium intybus,* sesquiterpene lactones, toxicity, rats.

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# INTRODUCTION

Since ancient times, natural plant-based products have been used to treat various diseases. The use of medicinal plants has received great attention recently, as they are considered a rich source of enormousbioactive components with various therapeutic effects (Jahani *et al.,* 2019).

*Cichorium intybus* (Chicory) is a Mediterranean plant of the Asteraceae family, widely cultivated in Europe, Asia, and Egypt. This plant is a terpene-rich source from the sesquiterpene lactones (STLs) group, along with other medicinally imperative compounds like coumarins, caffeic acid derivatives, fatty acids, alkaloids, amino acids, flavonoids, vitamins, and minerals (Satmbekova *et al.,* 2018).

*Cichorium intybus (C. intybus)* has diverse medicinal properties like anti-inflammatory, anticancer, antioxidant, antidiabetic, antimicrobial, antihyperlipid-emic, immunomodulatory, antinociceptive, hepatoprotective, neuro-protective, and cardioprotective properties. So, it is widely utilized for the treatment of different diseases like fever, jaundice, gout, hepatic diseases, splenic enlargement, and rheumatoid arthritis (Giambanelli *et al.,* 2018; Singh and Chahal, 2018; Salazar-Gómez *et al.,* 2020). According to recent studies, STLs and their derivatives can be responsible for these activities (Matos *et al.,* 2021; Jaśkiewicz *et al.,* 2022).

Despite the wide use of *C. intybus*, the toxicological data of its compounds are limited, as the studies in this aspect were concerned with the effect of *C. intybus* whole extract and didn't investigate the biological activities of those extracts' fractions (Schmidt *et al.,* 2007; Kuzina *et al.,* 2022).

Therefore, the current study aimed to evaluate the acute oral toxicity of *C. intybus* sesquiterpene lactones-enriched fraction in female Wistar rats, according to the Organization for Economic Cooperation and Development (OECD) guidelines 423. It would help in the establishment of toxicological data and recommend the minimum safety dose for this fraction. As far as we know, this is the first paper evaluating the acute toxicity of this fraction in Wistar rats.

# MATERIALS AND METHODS

## 1. Chemicals and kits

Methanol, formic acid, and acetonitrile (HPLC-grade) were bought from Sigma-Aldrich (USA). The ultrapure water was obtained from a Milli-Q water purification system (Millipore, USA). Commercial kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood hemoglobin (Hb), creatinine, and urea were bought from Vitro Scientific Company, Cairo, Egypt.

## 2. Plant material

*Cichorium intybus* leaves were bought from the Harraz® market. A voucher specimen (2023-BuPD 78) was placed at the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Egypt, and identified at the Department of Medicinal and Aromatic Plant, Faculty of Agriculture, Beni-Suef University.

## 3. Extraction and fractionation

The sample was dried and ground into a fine powder. The extraction was performed using ethanol: water (80:20, v/v) by ultra-sonication at room temperature for 15 minutes, followed by centrifugation for 10 minutes at 10,000 rpm. The collected supernatant was filtered, and the solvent was evaporated till dryness. The residue was extracted with dichloromethane (DCM) till exhaustion, followed by solvent evaporation till dryness. Before analysis, the extracted fraction was dissolved in methyl alcohol and filtered by a 0.22 µm membrane filter (Geetha *et al.,* 2012).

## 4. Liquid chromatography–mass spectrometry (LC–MS) of sesquiter-pene lactones-enriched fraction

**Instrumentation**

An Agilent 1290 Infinity II High Performance Liquid Chromatography (HPLC) system coupled with an Agilent 6470 triple quadrupole mass spectrometer (Agilent Technologies, USA) was used for analysis. It was equipped with an electro-spray ionization (ESI) source operating in positive mode. Data acquisition and processing were conducted by MassHunter software (Agilent Technologies, USA).

**HPLC Conditions**

Chromatographic separation was performed on an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm; Waters, USA). The mobile phase consisted of (A) water with (0.1%) formic acid and (B) acetonitrile with (0.1%) formic acid (Wang *et al.,* 2016). The gradient elution program is illustrated in Table 1. The flow rate was 0.3 mL $min^{-1}$, the column temperature was 30°C, and the injection volume was 5 µL.

**Table 1:** The gradient elution profile of HPLC

| B% | A% | Time (min) |
|---|---|---|
| 5 | 95 | 2-0 |
| 50 | 50 | 10-2 |
| 95 | 5 | 15-10 |

**Mass Spectrometry (MS) Conditions**

The MS was operated in positive electrospray ionization source (ESI) mode with the following settings: Scan range (m/z) 100–1000, 3.5 kV (Capillary voltage), 350°C (dissolving temperature), 10 L $min^{-1}$ (nebulizing gas ($N_2$) flow rate), and 20–40 eV (collision energy).

**5. The experimental design**

The experimental design was approved by the Institutional Animal Care and Use Committee Beni-Suef University (BSU-IACUC) with approval number 022-296. Thirty adult female Wistar rats, weighing 130–160 g were bought from the animal house of Al-Nahda University, Beni-Suef, Egypt. They were housed in cages under constant temperature and humidity, providing free access to feed and water. All animals were subject to an accommodation period of 14 days. The study was done following the OECD guideline 423 for the testing of chemicals (OECD, 2001). Owing to the safety of *C. intybus* whole extract, the limit test mentioned in the OECD guideline was followed. Thus, the chicory fraction at the limit dose (2000 mg/kg b.wt.) and a higher dose level (3000 mg/kg b.wt.) were used.

The animals are equally divided into three groups. The first group is control negative (control) treated with 2% tween80 (1mL/rat). The second (CF-I) and third (CF-II) groups received chicory sesquiterpene lactones-enriched fraction as a single dose of 2000, 3000 mg/kg. b.wt. dissolved in 2% tween80 and administered orally by gavages at a fixed volume of 1 mL/rat, respectively.

**Clinical observation and body weight**

Animal monitoring was performed individually after chicory fraction administration for morbidity, mortality, and any abnormal behavioral and physical variations periodically during the first day and then daily during the experimental period. Clinical observation for any toxic signs, including changes in fur, skin, eyes, and mucous membranes, convulsions, lethargy, somatomotor activity, salivation, and diarrhea. Body weight recording was on zero day before chicory fraction administration, and every week thereafter.

**Behavioral tests**

**The open field test:**

The open field maze is a wooden apparatus used for evaluating rat locomotor behavior (Gould *et al.,* 2009). The maze floor was divided into sixteen squares, as described by Brown *et al.* (1999). Five rats from each group were individually placed into one corner of the maze, and the rat behavior was video-recorded for 5 min. The maze was properly cleaned after each rat with 70% ethyl alcohol. The videos were displayed for behavioral analysis of the rats, as described by Choleris *et al.* (2001) and Kalueff and Tuohimaa (2004). Locomotion

(number of external squares crossed with all 4 paws), rearing (the frequency of rat standing against the maze's wall), and anxiety-like behaviors (freezing (immobilization time) /sec) were recorded.

**The elevated plus maze test (EPM):**
It is for measuring anxiety-like behaviors. It is an apparatus constructed from wood, placed 50 cm above the floor, and made up of two arms: the closed arm [(50 × 10 cm) and 30 cm sidewall height], the open arm (50 × 10 cm), and a central platform (10 $cm^2$) between arms. Each rat was separately placed at the end of the open arm, facing the central platform, and allowed to explore for 10 min. The total time and frequency spent by each rat in both arms was recorded by a videotaped camera (Komada *et al.,* 2008). The maze was properly cleaned with 70% ethyl alcohol after each rat.

**The Y-maze test:**
It is a Y-shaped maze for spatial short-term working memory measurement (Nasri *et al.,* 2012) through recording spontaneous alternative behavior percentage (SAP) in the maze, following Wall *et al.* (2004) and Rasoulijazi *et al.* (2007). Proper cleaning of the floor with 70% ethyl alcohol was performed after each rat.

**Sample collection**
Two weeks after chicory fraction administration, the rats were anesthetized with isoflurane for blood collection. Ethylene diamine tetraacetic acid (EDTA) was used as an anticoagulant for hematological assays, whereas for biochemical assays, plain tubes were used for serum separation.
All rats were humanely euthanized and subjected to gross pathological examination, and samples, including liver and kidney, were collected from each rat in 10% formalin solution for histopathological examination.

**6. Hematology and clinical biochemistry**
**Hematology**
Total erythrocyte and leukocyte counts were estimated by improved Haemocytometer (Feldman *et al.,* 2000). Packed cell volume (PCV) and blood hemoglobin (Hb) were determined as previously described by Thrall *et al.* (2004) and Drabkin and Austin (1932), respectively. Absolute and relative differential leucocytic counts were performed via Giemsa-stained blood smears from blood samples, as the method described by Jain (1993).

**Clinical biochemistry**
Hepatic transaminases were determined following the methodology of Reitman and Frankel (1957). Serum creatinine and urea were determined according to Bowers and Wong (1980) and Patton and Crouch (1977), respectively.

**7. Histopathology**
The hepatic and renal samples, after fixation in 10% formalin solution, were washed in water and dehydrated in ascending grades of alcohol, then in xylene. Samples were embedded in paraffin to prepare five μm paraffin sections, which stained with hematoxylin and eosin stain (H&E), as described by Bancroft and Layton (2012).

**8. Statistical analysis**
Data were presented as mean ± standard error (SE). Behavioral data expressed as means ± standard deviation (SD). Data analysis was done using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. The (P) value being less than 0.05 was statistically significant. SAP percentage was analyzed using the Kruskal-Wallis (non-parametric) test. IBM SPSS version 26 statistical software was used for all statistical analyses.

## RESULTS

**1. The LC-MS analysis of *C. intybus* sesquiterpene lactones-enriched fraction**
**Table 2** exhibits the phytoconstituents of *C. intybus* sesquiterpene lactones-enriched fraction. **Figure 1** shows the chromatogram for the LC-MS analysis and the pie chart for the relative percentage of identified compounds in the fraction.

**Table 2**: The identified phytoconstituents of *C. intybus* sesquiterpene lactones-enriched fraction via LC-MS analysis.

| Peak No. | Annotation | RT | m/z | Ion type | Relative percent | Molecular formula | Class |
|---|---|---|---|---|---|---|---|
| 1 | Dihydrolactucin | 2.44 | 277.05 | $[M-H]^-$ | 14.11 | $C_{15}H_{18}O_5$ | STLs |
| 2 | Caffeic acid | 4.54 | 180.05 | $[M]^+$ | 0.62 | $C_9H_8O_4$ | Phenolic acid |
| 3 | Lactucin | 6.12 | 276.2 | $[M]^+$ | 13.26 | $C_{15}H_{16}O_5$ | Sesquiterpene lactone |
| 4 | Chicoric acid | 7.02 | 474.11 | $[M]^+$ | 12.02 | $C_{22}H_{18}O_{12}$ | Phenolic acid |
| 5 | Chlorogenic acid | 8.00 | 354.09 | $[M]^+$ | 0.57 | $C_{16}H_{18}O_9$ | Phenolic acid |
| 6 | Dihydro-8-deoxylactucin (jaquinelin) | 8.37 | 262.28 | $[M]^+$ | 7.17 | $C_{15}H_{18}O_4$ | STLs |
| 7 | Scopolamine | 9.1 | 303.40 | $[M]^+$ | 4.02 | $C_{17}H_{21}NO_4$ | Alkaloid |
| 8 | Dihydrolactucopicrin | 10.40 | 412.15 | $[M]^+$ | 4.04 | $C_{23}H_{24}O_7$ | STLs |
| 9 | Lactucopicrin | 12.92 | 410.15 | $[M]^+$ | 8.15 | $C_{23}H_{22}O_7$ | STLs |
| 10 | Dihydrocostus lactone | 13.36 | 230.13 | $[M]^+$ | 3.17 | $C_{15}H_{18}O_2$ | STLs |
| 11 | Unidentified compound | 15.85 | 177.22 | ------- | 0.38 | ------- | ---------- |
| 12 | Esculetin | 17.98 | 178..05 | $[M]^+$ | 7.11 | $C_9H_6O_4$ | Coumarin |
| 13 | Luteolin | 19.39 | 286.04 | $[M]^+$ | 10.04 | $C_{15}H_{10}O_6$ | Flavonoid |
| 14 | Unidentified compound | 20.35 | ------- | ------- | 3.21 | --------- | ---------- |
| 15 | Apigenin | 22.01 | 270.07 | $[M]^+$ | 12.13 | $C_{15}H_{10}O_5$ | Flavonoid |

STLs, Sesquiterpene lactone

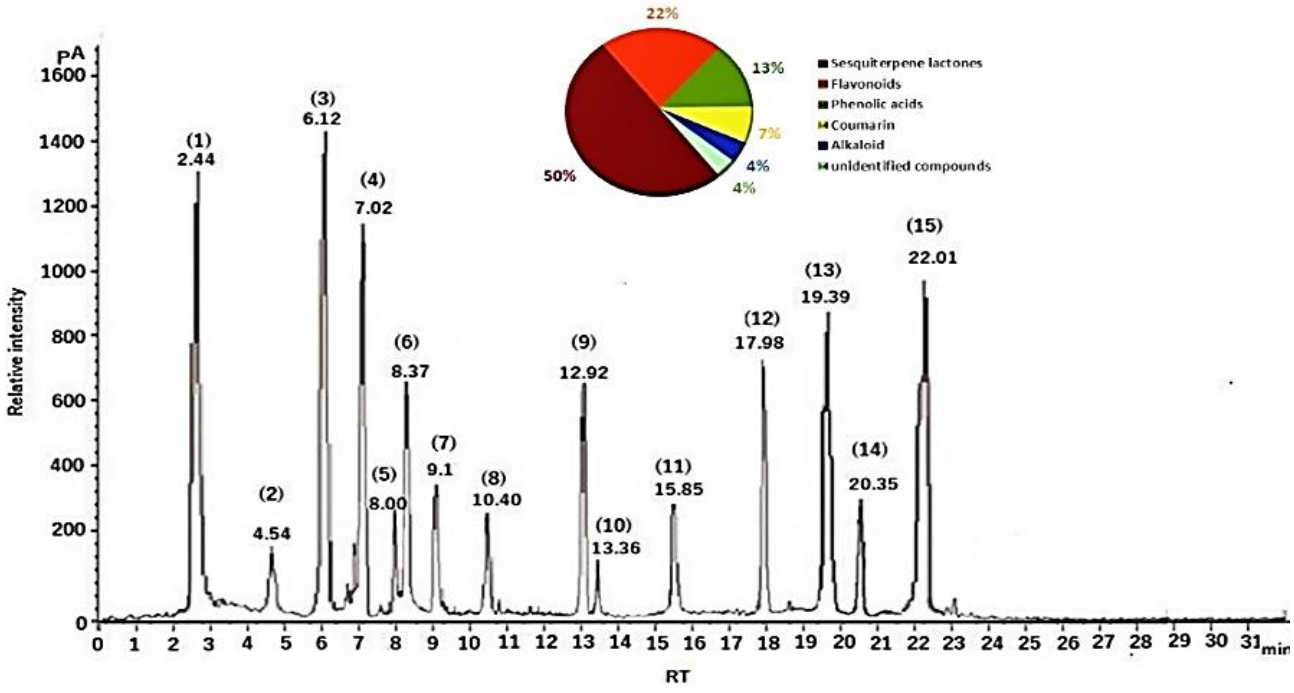


**Figure1:** The LC-MS chromatogram of *C. intybus* sesquiterpene lactones-enriched fraction and the relative percentage for identified compounds according to their classes.

## 2. Clinical observation and body weight

No abnormal clinical signs or mortalities were observed in the control and CF-I groups. For the CF-II group, there were depression, ruffled fur, a reduction in food intake, and diarrhea, with the death of 2 and 3 rats on the 3rd and 4th day after dosing, respectively. These symptoms were more intense in the first week after chicory fraction administration; thereafter, the severity reduced.

The weight gain of treated groups was markedly reduced from the control group in the first week after dosing; after that, no meaningful variations were detected **(Figure 2)**.

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## 4. Behavioral tests

**Table 3** illustrates the locomotor behavior of rats (the number of the crossed peripheral squares and rearing frequency). In the CF-I group, significant ($P<0.05$) elevation without significant alteration of immobilization duration (anxiety-like behavior) in the open field maze, compared to the control group. In addition, the time of immobilization significantly ($P<0.05$) increased without significant alteration in the rats' locomotion in the CF-II group related to the control group. The locomotor behavior was markedly elevated, and immobilization time was significantly reduced in the CF-I groups compared to the CF-II group.

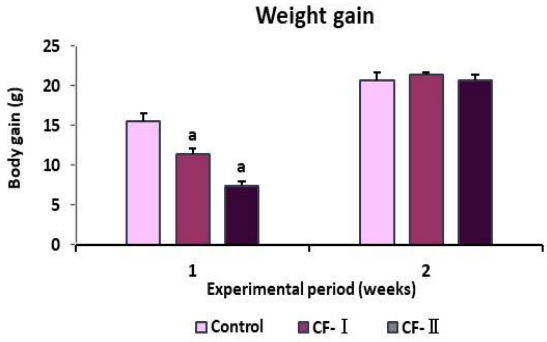


**Figure 2:** The weekly weight gains after a single oral treatment of *C. intybus* sesquiterpene lactones-enriched fraction. Data are presented as means ± SE
Control: control negative group
CF-I: Chicory fraction 2000 mg/kg b.wt.
CF-II: Chicory fraction 3000 mg/kg b.wt.
a= Significantly different from the control group.

**Table 3:** The effect of single oral treatment of *C. intybus* sesquiterpene lactones-enriched fraction on locomotor and anxiety-like behavior in the open field test.

| Behavior / Groups | Locomotor behavior | | Anxiety like behavior (immobilization time /sec) |
|---|---|---|---|
| | Number of crossed peripheral squares | Rearing frequency | |
| **Control** | 32.50 ± 3.22 | 2.25 ± 0.47 | 88.32 ± 9.7 |
| **CF-I** | 63.75 ± 11.08 [a] | 6.00 ± 1.82 [a] | 95±5.77 |
| **CF-II** | 20.00 ± 9.13 [b] | 2.75 ± 1.70 [b] | 162.50 ± 35.00 [ab] |

Data are presented as means ± SD (n=5) with dissimilar superscript letters (significantly differing at $P<0.05$): a) Significantly different from the control group; b) Significantly different from CF-I group. Control: Control negative group; CF-I: Chicory fraction 2000 mg/kg b.wt.; CF-II: Chicory fraction 3000 mg/kg b.wt.

**Table 4** shows that a prominent and significant (*P<0.05*) increment in the time rats spent in the open arms and a marked (*P<0.05*) decrease in the time spent in the closed arms of the elevated plus maze were observed in the CF-I group, compared to the control and CF-II groups. On the contrary, CF-II prolonged the time rats spent in the closed arm and shortened the time that spent the open arms significantly (*P<0.05*) related to the control group.

**Figure 3** declares that CF-I induced non-significant improvement in the short-term memory of rats compared to the control and CF-II groups.

**Table 4**: The effect of single oral treatment of *C. intybus* sesquiterpene lactones-enriched fraction on anxiety-like behavior in the elevated plus maze.

| Behavior / Groups | Open arm entrance frequency | Time spent in Open arm / sec | Closed arm entrance frequency | Time spent in Closed arm / sec |
|---|---|---|---|---|
| **Control** | 2.30 ± 1.20 | 76.00 ± 22.00 | 3.85 ± 0.90 | 162.50±18.90 |
| **CF-I** | 3.60 ± 0.55 | 120.00 ± 20.00 [a] | 3.60 ± 1.28 | 87.00 ± 18.2 [a] |
| **CF-II** | 3.00 ± 0.90 | 43.60 ± 13.00 [ab] | 2.40 ± 1.20 | 260.00 ± 55.9 [ab] |

Data are presented as means ± SD (n=5) with dissimilar superscript letters (significantly differing at *P<0.05*): a) Significantly different from control group; b) Significantly different from CF-I group. Control: Control negative group; CF-I: Chicory fraction 2000 mg/kg b.wt.; CF-II: Chicory fraction 3000 mg/kg b.wt.

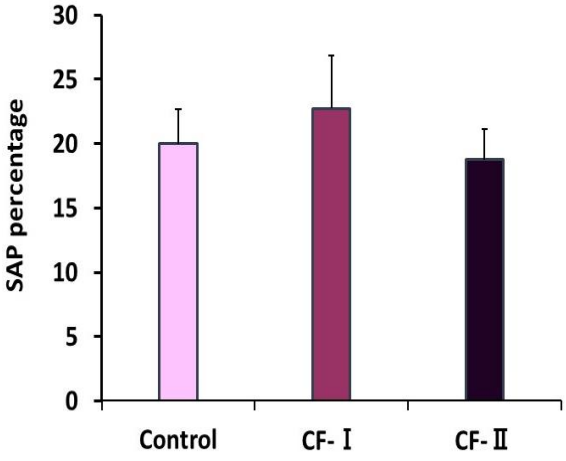


**Figure 3:** The effect of single oral treatment of *C. intybus* sesquiterpene lactones-enriched fraction on working memory of rats in the Y-maze. Data are presented as means ± SD
Control: Control negative group
CF-I: Chicory fraction 2000 mg/kg b.wt.
CF-II: Chicory fraction 3000 mg/kg b.wt.

## 5. Hematology and clinical biochemistry

For red blood cells, there were no statistically significant variations between experimental groups except for the Hb concentration; a significant decrease in its value was observed in the CF-II group compared to the control and CF-I groups.

For leucocytes, a significant leucopenia accompanied by absolute lymphopenia was observed in the CF-I and CF-II groups in comparison with the control group. The CF-II group showed absolute eosinophilia compared with the control and CF-I groups **(Table 5)**.

The activities of ALT and AST were markedly reduced in the serum of both treated groups, with no significant changes in values of creatinine and urea, compared with the control group (**Figure 4)**.

**Table 5**: The effect of single oral treatment of *C. intybus* sesquiterpene lactones-enriched fraction on various hematological parameters.

| Groups / Parameters | Control | CF-I | CF-II |
|---|---|---|---|
| **Erythrocyte (×10$^6$/µL)** | 7.17 ± 0.30 | 6.96 ± 0.41 | 6.16 ± 0.09 |
| **PCV %** | 42.33± 1.20 | 42.33± 0.33 | 39.33 ±0.33 |
| **Hb (g/dl)** | 11.83 ± 0.27 | 11.53 ± 0.09 | 10.63 ± 0.22 [ab] |
| **Leucocyte (×10$^3$/µL)** | 14.18 ± 0.15 | 11.22 ± 0.23 [a] | 12.45 ± 0.26 [a] |
| **Lymphocyte (×10$^3$/µL)** | 11.72 ± 0.25 | 8.89 ± 0.15 [a] | 8.69 ± 0.25 [a] |
| **Neutrophil (×10$^3$/µL)** | 1.23 ± 0.12 | 1.12 ± 0.08 | 2.21 ± 0.51 |
| **Eosinophil (×10$^3$/µL)** | 0.47 ± 0.05 | 0.49 ± 0.04 | 0.68 ± 0.02 [ab] |
| **Monocyte (×10$^3$/µL)** | 0.76 ± 0.04 | 0.71 ± 0.05 | 0.87 ± 0.02 |

Data are presented as means ± SE (n=5) with dissimilar superscript letters (significantly differing at $P < 0.05$): a) Significantly different from the Control group; b) Significantly different from CF-I group. Control: Control negative group; CF-I: chicory fraction 2000 mg/kg b.wt.; CF-II: chicory fraction 3000 mg/kg b.wt.; PCV: packed cell volume; Hb: hemoglobin.

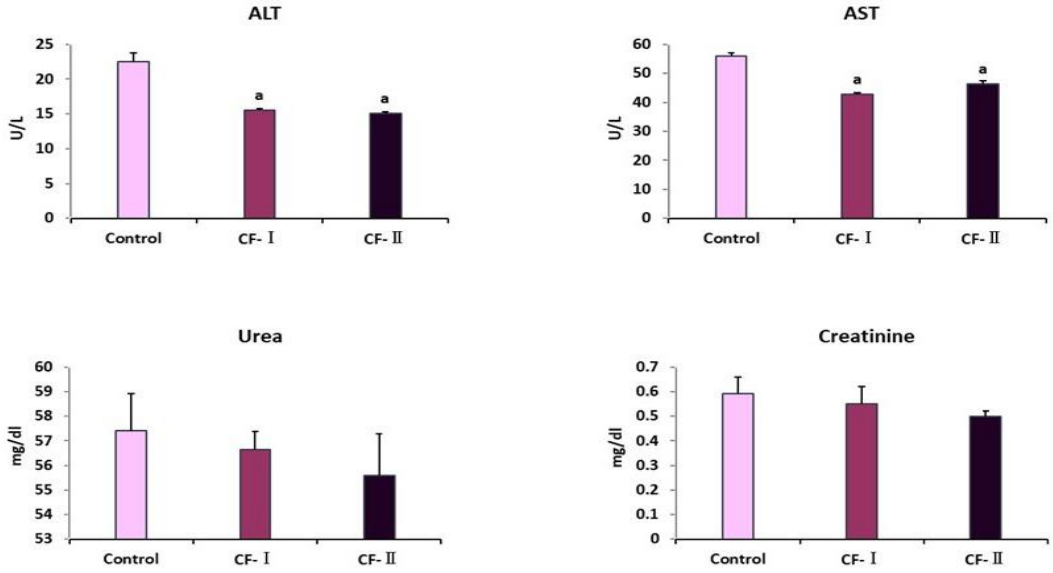


**Figure 4:** The effect of single oral treatment of *C. intybus* sesquiterpene lactones-enriched fraction on different biochemical parameters. Data are presented as means ± SE (n=5) with dissimilar superscript letters (significantly differing at $P<0.05$): a) Significantly different from the control group. Control: Control negative group; CF-I: chicory fraction 2000 mg/kg b.wt.; CF-II: chicory fraction 3000 mg/kg b.wt.; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

## 6. Histopathological results

The histopathological pictures of the liver and kidney in different experimental groups are presented in Figure 5. The H&E-stained sections of hepatic tissues for normal rats showed normal hepatic architecture (Figure 5, A). Conversely, the hepatic tissues of rats in the CF-I group (2000 mg/ kg b.wt.) revealed congestion of the hepatic vasculature and moderate lymphocytic infiltration within the portal triads and intra-lobular (Figure 5, B). While rats in the CF-II group (3000 mg/ kg b.wt.) showed massive mononuclear infiltration, including eosinophils within portal triads and intra-

lobular in a focal and diffuse manner, and severe congestion (Figure 5, C). Along with kidneys from normal rats, they showed normal histology of renal tissue (renal tubules and glomeruli) (Figure 5, D). The stained renal tissues of the CF-I group (2000 mg/ kg b.wt.) showed features of nephrosis (vacuolation and hyaline droplets) and mild congestion (Figure 5, E). The rats of the CF-II group received 3000 mg/kg b.wt. showed features of nephrosis in the form of vacuolation and hyaline droplets, with several proximal convoluted tubule necrosis, glomerular atrophy, mild fibrosis, and thickening of the basement membrane (Figure 5, F).

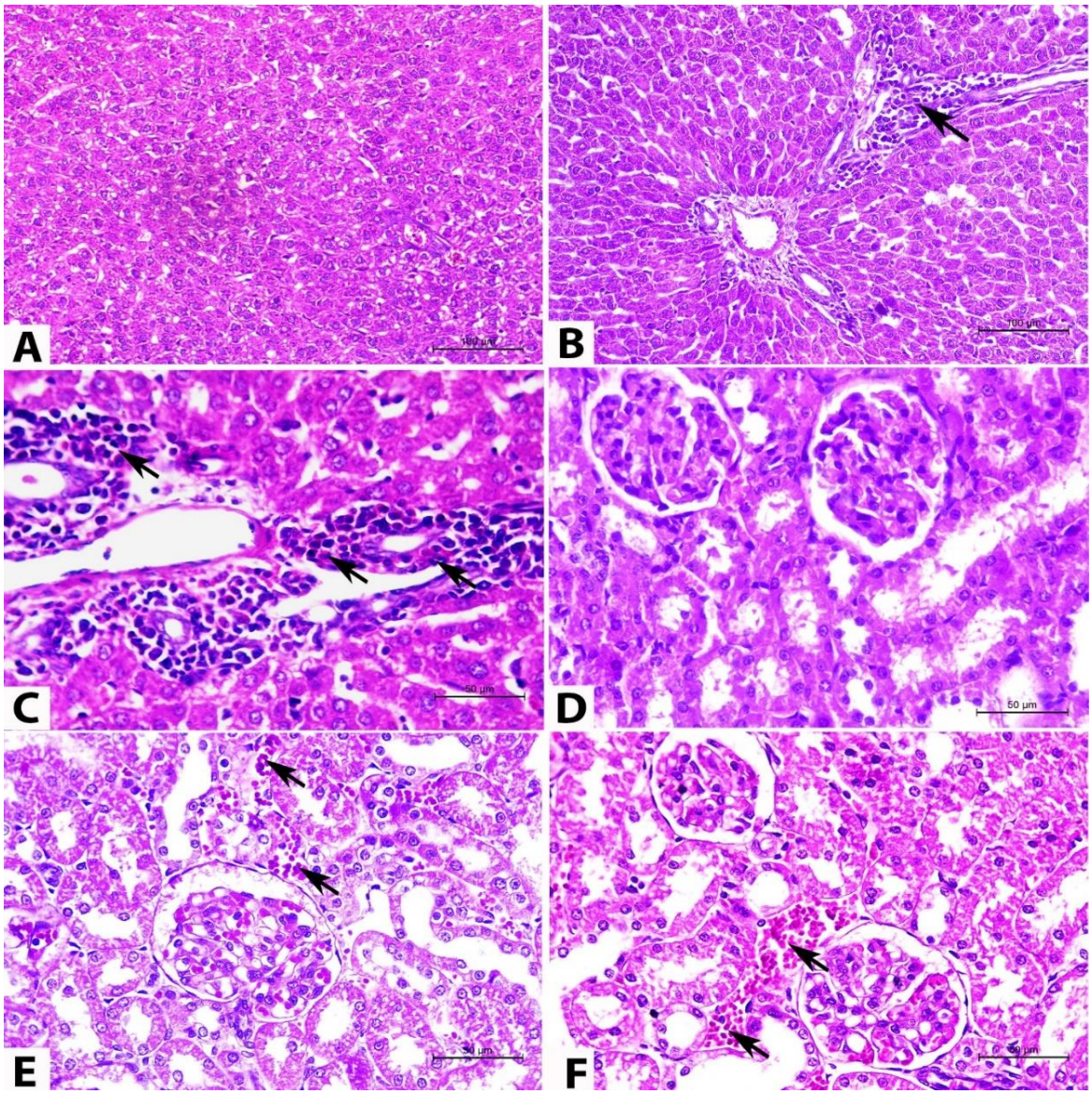


**Figure 5:** Photomicrographs of representative hepatic and renal tissue samples from rats. (A) Normal rats showed normal hepatic architecture. (B) Livers of rats treated with 2000 mg/kg b.wt. (CF-I) showed dilatation of hepatic sinusoids and moderate intralobular lymphocytic infiltration (arrow). (C) Rats in the CF-II group (3000 mg/kg b.wt.) showed massive mononuclear cells and eosinophils infiltration within portal triads and intralobular (arrow). (D) Renal tissues from normal rats (Control) showed normal histological architecture characterized by renal tubules and glomeruli. (E) The kidneys of rats in the CF-I group (2000 mg /kg b.wt.) revealed features of nephrosis (vacuolation and hyaline droplets) and congestion (arrow). (F) Rats in the CF-II group (3000 mg/kg b.wt.) exhibited features of nephrosis (vacuolation and hyaline droplets), congestion (arrow), and basement membrane thickening (H&E, x200, x400).

# DISCUSSION

Medicinal plants have received great attention in recent years. *Cichorium intybus* is one of the broadly distributed members of the Asteraceae family. It has been utilized as a traditional herbal remedy for numerous illnesses. This paper aimed to assess the *in vivo* toxicity of *C. intybus* sesquiterpene lactones-enriched fraction in Wistar rats after a single oral administration at the dose of 2000 and 3000 mg/kg b.wt.

Determining the changes in body weight and weight gain is a part of evaluating the safety of herbal extracts. In the present study, there was a prominent reduction in weight gain in rats receiving chicory fraction at both doses only in the 1st week, but it returned to normal in the 2nd week of the study. Our results are consistent with a recent study by Egbule *et al.* (2023), who stated that the oral administration of STLs-rich fraction of *Tithonia diversifolia* in rats at a dose of 2000 mg/kg/day for 28 days induced a significant reduction in weight of rats in the first week of the study that normalized during the following weeks of the study. This decrease in weight gain may be caused by the gastrointestinal irritation caused by STLs rich fraction administration, leading to decreased appetite and feed intake as STLs are mucosal irritants, and the majority of them are thought to have a gastro-irritant action (Schmidt, 1999; Amorim *et al.,* 2013).

Evaluation of hepatic and renal functions, along with hematological parameters, plays a vital role in toxicological studies, as they provide numerous functions that warrant the organism's survival, and their alterations are thought to be a reliable indicator of toxicity in people or animals (Arika *et al.,* 2016; Al-Afifi *et al.,* 2018).

In our study, the reduction in the blood Hb concentration in rats receiving 3000 mg/kg b.wt., along with the reduction in the ALT and AST activities at both doses compared to the control group. The previous reports by Amorim *et al.* (2013) and Karadeniz *et al.* (2021) mentioned that due to the alkylating ability of STLs, they can easily react with the SH group of proteins and enzymes, leading to functional variations of alkylated proteins.

Leucocytes are the primary defense against pathogens and play a fundamental role in innate immunity, inflammatory responses, and tissue damage (Chen *et al.,* 2018; Marshall *et al.,* 2018). Leucopenia accompanied by lymphopenia in rats receiving chicory fraction might be due to lymphocytic infiltration in the hepatic tissue, as shown in the histopathological examination, leading to a reduction in circulating lymphocytes. Ramos *et al.* (2015) evaluated the hepatotoxic effect of D-Limonene, a monoterpene present in different plants, and found that there was an influx of T cytotoxic (CD8+) and (CD3+) lymphocytes in liver tissue by immunohistochemistry. Hepatotoxic metabolites from D-limonene may be responsible for this influx, which stimulates specific immune reactions. Therefore, the potential for direct leucocytic aggression is believed to be the metabolism of STLs, which produced potentially harmful substances that affected mitochondrial function and triggered particular immune responses.

On the other hand, the rats receiving the highest dose of chicory fraction showed eosinophilia, escorted by eosinophilic infiltration in the hepatic tissue, with 50% death of the rats in this group, which may be due to treatment-related allergic reactions. The majority of STLs are alkylant STLs that act as haptens, which can react with the SH group of proteins, forming a hapten-protein complex. This complex can be recognized by the immune system, producing undesirable hyper-sensitivity reactions (Amorim *et al.,* 2013). Das *et al. (*2016) stated that the contact with chicory roots or leaves

induced allergic reactions, such as facial erythema, skin irritation, chronic eczema, and bronchospastic reactions. Additionally, chicory causes oral allergy syndrome in persons with birch pollen allergies (Cadot *et al.,* 2003), and it can induce anaphylactic type I allergy, but in scarce cases (Morita *et al.,* 2007; Willi *et al.,* 2009; Denisow-Pietrzyk *et al.,* 2019).

The renal lesions, accompanied by chicory fraction administration in our study, are in line with the previous studies of De Oliveira *et al.* (2011) and Passoni *et al.* (2013), who assessed the toxicity of STLs-enrich extracts of *Smallanthus sonchifolius* and *Tithonia diversifolia*, respectively, in a repeated-dose toxicity study for 90 days in Wistar rats, confirming the toxicity of STLs. As the observed changes depended on the dose and duration of treatment, the severity of renal pathology was more intense, with the highest dose in the current study.

In addition to evaluating the effect of the chicory fraction on general health, we also closely monitored behavioral pattern changes. The open field and Y-maze tests are commonly used to evaluate locomotor activity, anxiety, and short-term memory in rats (xu *et al.,* 2017).

The observed behavioral alterations in the elevated plus maze test indicated that CF-I had an anxiolytic effect; however, the higher dose caused an anxiogenic action. Some early reports studied *C. intybus* extract and its active principles on the behavior of laboratory animals as human models. For example, the methanolic extract of *C. intybus* at a dose of 500 mg/kg b.wt. induced an anxiolytic effect without disturbance of spontaneous movement of mice. This efficacy is due to the coumarins and flavonoids in the extract (Tripathy and Neeraja, 2020). In addition, intraperitoneal injection of 50 mg/kg b.wt. of chicory extract decreased the neuropathy induced by pyridoxine in rats, which appeared as increased spontaneous movement (Hasannejad *et al.,* 2019). In our study, the anxiolytic effect of chicory fraction is believed to be thankful to esculetin (coumarin), luteolin (flavonoid), and Apigenin (flavonoid) in the used fraction.

The obtained anxiogenic (increased anxiety) response with the highest dose may be attributed to STLs-induced neurotoxicity. The STLs, mainly found in the Asteraceae family, have a neurotoxic effect via antagonizing gamma-aminobutyric acid (GABA) receptors, leading to increased anxiety-like behaviors (Amorim *et al.,* 2013; Liu *et al.,* 2018).

# CONCLUSION

*Cichorium intybus* sesquiterpene lactones-enriched fraction at 2000 mg/kg b.wt., as a single oral dose, had an anxiolytic effect with non-significant memory enhancement efficacy. It induced mild to moderate hemato-biochemical and histopathologic alterations. On the other hand, the higher dose had an anxiogenic effect and 50% mortality of rats in the first week after dosing. The remaining surviving rats showed marked hemato-biochemical and histopathological alterations. Therefore, the *Cichorium intybus* sesquiterpene lactones-enriched fraction is safe as a single dose up to 2000 mg/kg b.wt., and the 3000 mg/kg b.wt. oral dose induced marked toxic effects.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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# دراسة السمية الحادة للجزء الغنى ب لاكتونات السيسكيتربين من نبات الهندباء في الفئران

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اصبح لنبات الهندباء مجموعة واسعة من الاستخدامات العلاجية. تهدف هذه الدراسة إلى تقييم السمية الحادة للجزء الغنى ب لاكتونات سيسكيتيربين من نبات الهندباء في الفئران. تم تقسيم ثلاثين فأرًا بالتساوي إلى ثلاث مجموعات. المجموعة الأولى غير معالجة (الضابطة). تلقت المجموعتان الثانية والثالثة جرعة واحدة من الجزء الغنى ب لاكتونات سيسكيتيربين من نبات الهندباء بجرعات ٢٠٠٠، ٣٠٠٠ ملجم/كجم من وزن الجسم، على التوالي. تمت ملاحظة الفئران خلال فترة التجربة وتسجيل الوفيات والعلامات السريرية الغير الطبيعية، بالإضافة إلى وزن الجسم أسبوعيًا. وفي نهاية التجربة تم إجراء الاختبارات السلوكية وجمع عينات الدم لاختبارات الدم والكيمياء الحيوية. كما تم جمع أنسجة الكبد والكلى للفحص الباثولوجي. في الأسبوع الأول من التجربة، كان هناك انخفاض كبير في وزن الجسم في كلا المجموعتين المعالجتين وموت نصف الفئران التي تلقت ٣٠٠٠ ملجم/كجم من وزن الجسم. كان للجرعة ٢٠٠٠ ملجم/كجم من وزن الجسم تأثير مزيل القلق وزيادة النشاط الحركي. في حين أن أعلى جرعة كان لها تأثير مزيد للقلق. كان هناك نقص في خلايا الدم البيضاء مصحوب بنقص مطلق للخلايا الليمفاوية وانخفاض في نشاط انزيمات الكبد في الدم في كلتا الجرعتين. من ناحية أخرى تم رصد ارتفاع ملحوظ لخلايا الايزينوفيل في الفئران التي تلقت أعلى جرعة. لذلك الحد الأقصى للجرعة الآمنة من الجزء الغنى ب لاكتونات سيسكيتيربين من نبات الهندباء هو ٢٠٠٠ ملجم/كجم من وزن الجسم ، حيث له تأثير مُضادّ للقلق. بينما الجرعة الأعلى (٣٠٠٠ ملجم/كجم من وزن الجسم) كان لها تأثير مُثير للقلق، ونسبة نفوق ٥٠٪ من الفئران، مما يُشير إلى تأثير سُمّي ملحوظ.

**الكلمات المفتاحية**: الهندباء، لاكتونات سيسكيتيربين، السمية، الفئران.