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ACUTE SAFETY STUDY OF *SALVIA OFFICINALIS* TERPENOIDS RICH FRACTION IN WISTAR RATS

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ABSTRACT

Salvia plants become more popular due to their health-promoting and antioxidant potentials. The present study aimed to investigate the safety limit of Salvia officinalis terpenoids rich fraction in Wistar rats. Thirty adult female rats were divided equally into three groups. The first one was control. The second and third groups received Salvia fraction as a single oral dose of 2 and 3 g/kg b.w, respectively. During the experimental period, the rats were observed for any abnormal clinical signs, morbidity and mortality as well as body weight gain was weekly recorded. At the end of the experiment, behavior tests were performed and blood specimens were collected for hematological and biochemical examinations. The organs (liver and kidney) were collected for histopathology. There were no abnormal variations in clinical signs or weight gain at both doses. Salvia fraction at both doses had a distinct anxiolytic effect; however, it improved working memory only at 2 g/ kg b.w dose. There was an increment in locomotor activity in rats receiving 3 g/kg b.w. For hematology and clinical biochemistry, there was mild leucopenia accompanied by absolute lymphopenia and a decrease in ALT and AST activities in rats receiving 3 g/kg b.w of Salvia fraction, besides no significant change in urea and creatinine in both treated groups. In conclusion, *Salvia officinalis* terpenoids rich fraction appears to have no adverse toxic effects, except for minimal alterations in leucogram, liver enzymes, and hepatic and renal tissues induced in rats treated with 3 g/kg b.w of Salvia fraction.

***Key words**: Salvia officinalis*, terpenoids, safety, rats.

INTRODUCTION

Today, the plants are widely used as traditional medications due to their massive benefits to humans and animals. Their active components are widely utilized in the pharmaceutical industry as they are the original source of most medications. The utilization of herbal remedies has received attention in recent years because of their wide range of biological activity, accessibility, affordability, and safe use (Shivaraj *et al.*, 2021).

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Salvia officinalis (also known as common sage), one of the most widely distributed members of the Lamiaceae family, has been used as a traditional herbal remedy against different diseases such as hyperglycemia, ulcers, dizziness, gout, rheumatism, diarrhea, and paralysis (Rhaimi *et al.*, 2023).

Phytochemical analysis for leaf extracts of *S. officinalis* reveals that they consist of various active components, mainly mono- and diterpenes exhibiting numerous activities: antibacterial, antifungal, antiprotozoal, anti-inflammatory, antioxidant, antimutagenic, and cytotoxic activities. (Llurba-Montesino and Schmidt, 2018; Li *et al.,* 2019; Salamatin *et al.*, 2020; Rhaimi *et al.,* 2023).

Despite the wide use of *S. officinalis* leaf extracts, the toxicological information of its components is limited. Most of the studies that have been done in this aspect were interested in the effects of *S. officinalis* whole extract or essential oil (Rhaimi *et al.*, 2023; Bouteldja *et al.,* 2023), while the toxicity or safety threshold for fractions of those extracts is lacking.

Hence, this study aimed to evaluate the acute oral safety limit of *S. officinalis* terpenoids rich fraction in Wistar rats following the Organization for Economic Cooperation and Development (OECD) guidelines 423 (OECD, 2001), which would help in establishing toxicological data and suggesting the minimal safety level of this fraction.

MATERIALS AND METHODS

1. Chemicals and kits

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), hemoglobin (Hb), urea, and creatinine kits were purchased from Vitro Scient Company, Cairo, Egypt.

2. Plant material

Salvia officinalis leaves were purchased from Harraz® Market in August 2023. A voucher specimen (2023-BuPD 77) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Egypt.

3. Extraction and fractionation

The extraction was performed as the method described by Al Musayeib *et al.* (2014) with few modifications. The powdered plant (1.5 kg) was extracted with 80% ethanol till exhaustion by the aid of sonication for three intervals of 20 min each. The combined ethanolic extract was concentrated under vacuum to provide a final dry residue of 157g. The residue was then suspended in 500 ml distilled water and subjected to fractionation using ethyl acetate till exhaustion to obtain 80 g of terpenoids rich fraction. One gram of this fraction was stored in closely tight container for LC-MS study.

4. Liquid chromatography–mass spectrometry (LC–MS) analysis of *S. officinalis* terpenoids rich fraction

LC-MS Instrumentation and Conditions

For the separations, gradient of mobile phase A (2 v/v% formic-acid in methanol: water (7:93)) and mobile phase B (2 v/v% formic-acid in methanol) was used. The gradient profile was configured as follows: 0.00 min 0% B eluent, 10.00 min 90% B eluent, 10.01 min 0% B eluent and 15.00 min 0% B eluent. The flow rate was 0.2 mL /min; the temperature of column was ambient and its outlet was connected to the inlet of electrospray sample. The electrospray source was worked in positive and negative modes and the interface conditions were as follows: CDL voltage of 5 V, capillary voltage of 5 kV, deflector voltage of 40 V and CDL temperature of 250 °C. The flow of de-solvation gas was 3.5 L /min that obtained from a nitrogen gas generator. The voltage of detector was 2.0 kV (Boros et al., 2010).

Apparatus

An Agilent series 1200 HPLC system (Agilent Technologies) equipped with a

G1367D auto-sampler, G1312B binary pump, a G1379B degasser, and a G1316B column compartment thermostat. The system of HPLC was connected to an Agilent quadrupole triple mass spectrometer (6410 B) that coupled to a source of electrospray ionization (ESI+). A Zorbax XDB C18 column (50x4.6 mm and 1.8 µm particle size) from Agilent was used for the separation. The mobile phase composition was equilibrated during 2 min and the stationary phase returned to the initial conditions within 2 min. The running time totally was 17 minutes. The volume of injection was 5 µL, and column temperature was kept at 25 ºC. The values pf ESI source were as follows: drying gas (nitrogen) temperature 350 °C with flow rate 10 L/min, capillary voltage 3500 V and nebulizer pressure 40 psi. The detection was achieved using multiple reactions monitoring mode (MRM). The Agilent MassHunter software was for mass spectral analysis. (Vuković *et al.*, 2020).

5. Experimental design

Thirty adult healthy Wistar female rats weighing 140–170 g were purchased from Al-Nahda University animal house, Beni-Suef, Egypt. They were housed in plastic cages under constant humidity and temperature (25 ± 2 °C) with free access for water and feed. All rats were subject to a two-week acclimatization period before the experiment. This study was performed following the OECD guideline no. 423 for testing of the chemicals (OECD, 2001). Based on the high safety of *S. officinalis* whole extract and essential oil, the limit test described in the OECD guideline was followed. Therefore, the fraction at the limit dose (2 g/kg b.w) and a higher dose level (3 g/ kg b.w) were used. The animals were divided into three groups (10 rats in each group). The 1st group was negative control (Control) treated orally with 2% tween80 (1 ml/rat). Salvia terpenoids rich fraction at the dosage of 2 and 3 g/kg b.w was dissolved in 2% tween80 and administered by gavages at a fixed volume of 1 ml/rat as a single dose for the 2nd (SF-I) and 3rd (SF-II) groups, respectively. This experimental protocol was approved by the Institutional Animal Care and Use Committee of Beni-Suef University (BSU-IACUC) with approval number 022-296.

Clinical observation and body weight

All rats were observed individually for morbidity or mortality throughout the first thirty minutes after Salvia fraction administration, periodically during the first twenty-four hours, and then every day throughout the experimental period. Clinical observation included any atypical physical and behavioral changes or toxic signs. Individual body weight of all animals was determined on zero day shortly before Salvia fraction administration and weekly thereafter.

Neurobehavioral tests

Measuring locomotor behavior [open field test]:

The open field maze is an apparatus designated to evaluate locomotor behavior and exploration of rats (Gould *et al.*, 2009). It is constructed of wood; the floor was divided into 16 squares as previously described by Brown *et al.* (1999). From each group, five rats were individually paced into one of the four corners of the maze. Then after the behavior of each rat was video recorded for five min. The proper cleaning of the maze was performed after each rat using 70 % ethyl alcohol. The recorded videos were displayed for analysis of the rats' behavior following Choleris *et al.* (2001) and Kalueff and Tuohimaa (2004). Locomotion (number of outside squares crossed with all four paws and rearing; the frequency with which rat stood against maze's wall), anxiety like behaviors [freezing (immobility time) /sec], and exploration (number of center squares entries with all four paws and time the rat spent in them) were recorded.

Measuring of anxiety- like behavior [elevated cross maze test (EPM)]:

It is a wooden-constructed apparatus consists of two opposing arms: the closed

arm (50 × 10 cm), 30 cm sidewall height, and the open arm (50 × 10 cm). There is a central platform (10 cm^2) between arms. It was placed 50 cm above the floor. The rats were separately placed at the end of the open arm, facing the central platform, and allowed to explore the apparatus for 10 min. The frequency with the total time rats spent in closed and open arms were recorded by a videotaped camera (Komada *et al.*, 2008). The maze floor was cleaned using 70% ethyl alcohol after each individual rat.

Measuring working memory [Y-maze test]:

This is a Y-shaped maze for measuring spatial short-term working memory (Nasri *et al.*, 2012) via recording of spontaneous alternative behavior percent (SAP) in arms of the maze following the methodology of Wall *et al.* (2004) and Rasoolijazi *et al.* (2007). The floor of arms was cleaned using 70% ethyl alcohol after each individual rat.

Sample collection

After the end of the experimental period (day 15), blood was collected from the retro-orbital plexus of animals for hematology and clinical biochemistry. Ethline diamine tetra acetic acid (EDTA) was used as an anticoagulant for hematological samples. Blood samples were collected on plain tubes for serum separation.

All rats were subjected to gross pathological examination and the specimens from the liver and kidney were collected from each animal and preserved in 10% formalin for microscopical examination.

6. Estimation of hematological and biochemical parameters

Hematological analysis

Determination of total erythrocytic and leucocytic counts was done using an improved Haemocytometer in accordance with Feldman *et al.* (2000). Packed cell volume (PCV) was determined as described by Thrall *et al.* (2004) and the estimation of blood hemoglobin (Hb) was performed by the colorimetric cyanomethemoglobin technique in accordance with Drabkin and Austin (1932) using commercially available kits.

Differential leucocytic count was accomplished manually; thin blood smears were made from each blood sample, and then they were stained with Giemsa stain. Calculation the values of relative and absolute differential leucocytic counts was performed according to the method described by Jain (1993).

Biochemical analysis

The activity of liver enzymes (ALT and AST) in serum samples was determined as previously reported by Reitman and Frankel (1957) using commercially available kits.

Urea and creatinine were measured colorimetrically in accordance with Patton and Crouch (1977) and Bowers and Wong (1980), respectively, following the instructions of commercial kits.

7. Histopathological examination

The tissue samples collected in 10% formalin (liver and kidney) were prepared and stained with hematoxylin and eosin stain (H&E) as previously described by Bancroft and Layton, 2013.

8. Statistical analysis

Data were exhibited as mean ± standard error (SE). Data analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A P value that was lower than 0.05 was considered statistically significant. SAP percentage was analyzed using Kruskal-Wallis (non-parametric) test.

All statistical analyses were performed using IBM SPSS version 26 statistical software. The degree of significance was represented on each graph by stars: ** $p < 0.01$ (very significant) * $p < 0.05$ (significant).

RESULTS

1. LC-MS analysis of *S. officinalis* terpenoids rich fraction

The phytoconstituents of the *S. officinalis* terpenoids-rich fraction were presented in **Table 1**.

Figure 1 exhibited the chromatogram obtained from LC-MS analysis with the pie chart for the relative percentage of identified compounds in the fraction.

Table 1: Identified phytoconstituents of *S. officinalis* terpenoids-rich fraction according to LC-MS analysis.

Peak No.	Annotation	RT	m/z	Ion type	Relative percent	Molecular formula	Class
1	Gallic acid	0.865	169.000	$[M-H]^-$	15.045	$C_7H_6O_5$	Phenolic acid
2	Catechin	1.664	289.056	$[M-H]^-$	1.212	$C_{15}H_{14}O_6$	Tannin
3	Caffeic acid hexoside	2.311	340.08	$[M]^+$	1.471	$C_{15}H_{18}O_9$	Phenolic acid
4	Luteolin-7-O-rutinoside	4.343	286.221	$[M]^+$	0.684	$C_{27}H_{30}O_{16}$	Flavonoid
5	Ferulic acid	4.728	216.000	$[M+Na]^+$	1.321	$C_{10}H_{10}O_4$	Phenolic acid
6	Luteolin-7-O-glucoside	5.106	448.100	$[M]^+$	0.518	$C_{21}H_{20}O_{11}$	Flavonoid
7	Apegenin-7-O-glucoside	5.803	432.001	$[M]^+$	1.266	$C_{21}H_{20}O_{10}$	Flavonoid
8	Rhamnetin	7.211	315.121	$[M+H]^+$	5.101	$C_{16}H_{12}O_7$	Flavonoid
9	Cirsimaritin	8.321	315.121	$[M+H]^+$	0.781	$C_{17}H_{14}O_6$	Flavonoid
10	Pectolinarigenin	9.422	315.072	$[M+H]^+$	3.121	$C_{17}H_{14}O_6$	Flavonoid
11	Epiisorosmanol	9.721	345.000	$[M-H]^-$	8.635	$C_{20}H_{26}O_5$	Diterpene
12	Rosmanol isomer	10.165	345.000	$[M-H]^-$	3.818	$C_{20}H_{26}O_5$	Diterpene
13	Rosmaridiphenol	12.864	340.080	$[M+Na]^+$	0.352	$C_{20}H_{28}O_3$	Diterpene
14	Romansol methyl ether	10.812	359.187	$[M-H]^-$	11.377	$C_{21}H_{28}O_5$	Diterpene
15	Rosmanol methyl ether isomer	11.634	359.188	$[M-H]^-$	18.028	$C_{21}H_{28}O_5$	Diterpene
16	Asiatic acid	11.008	489.056	$[M+H]^+$	3.788	$C_{30}H_{48}O_5$	Triterpene
17	Rosmaridiphenol isomer	13.752	340.010	$[M]^+$	0.712	$C_{20}H_{28}O_3$	Diterpene
18	Carnosol	14.161	329.550	$[M-H]^-$	3.365	$C_{20}H_{26}O_4$	Diterpene
19	Carnosic acid	22.81	331.188	$[M-H]^-$	4.212	$C_{20}H_{28}O_4$	Diterpene
20	12-Methoxy carnosic acid	28.903	345.210	$[M-H]^-$	0.695	$C_{21}H_{30}O_4$	Diterpene
21	Ursolic acid	33.022	455.319	$[M-H]^-$	10.076	$C_{30}H_{48}O_3$	Triterpene
22	Micromeric acid	34.224	453.213	$[M-H]^-$	4.422	$C_{30}H_{46}O_3$	Triterpene

2. Clinical observation and body weight

No abnormal clinical signs were observed in rats treated with *S. officinalis* fraction at both doses during the experimental period when compared with the control group.

Comparing weekly weight gain during the experimental period in *S. officinalis* fraction groups with that of vehicle control, no meaningful variations were detected (Figure 2).

3. Neurobehavioral tests

Table 2: Effect of single oral treatment of *S. officinalis* terpenoids rich fraction on locomotor and anxiety-like behaviors.

Behavior / groups	Locomotor behavior		Exploratory behavior (Time rats spent in central squares/sec)	Anxiety like behavior (Freezing time /sec)
	Number of crossed peripheral squares	Rearing frequency		
Control	32.50 ± 3.22 [a]	2.25 ± 0.47 [a]	0.001 ± .0001	83.75 ± 8.98
SF-I	50.00 ± 7.07 [ab]	6.50 ± 1.55 [ab]	2.00 ± 1.08	70.00 ± 9.12
SF-II	52.50 ± 6.614 [b]	16.25 ± 4.32 [b]	0.001 ± .0001	76.25 ± 10.28

Data are expressed as means ± SE (n=5) with dissimilar superscript letters in each column (significantly differing at $P < 0.05$). Control: control negative group; SF-I: Salvia fraction 2 g/kg b.w; SF-II: Salvia fraction 3 g/kg b.w.

Table 2 declared that the highest dose of Salvia fraction significantly ($p<0.05$) increased locomotor behavior of rats, including the number of crossed peripheral squares and rearing frequency in the open field maze.

Figure 3 and 4 elucidated the effect of *S. officinalis* terpenoids rich fraction on anxiety-like behavior and working memory.

Data described by Figure 3 indicated that the Salvia fraction at both doses significantly ($p<0.01$) increased the time/sec that rats spent in the open arm of EPM in comparison with the control group. However, in the SF-I group, there was a significant ($p<0.01$) reduction in the time/sec rats spent in EPM closed arm in a more significant ($p<0.05$) manner than that recorded in the SF-II group when compared to the control group and at $p<0.01$ in comparison with rats in the SF-II group.

Figure 4 revealed that the lower dose of Salvia fraction (2 g/kg b.w) increased working memory significantly ($p<0.01$), while the highest one (3 g/kg b.w) induced non-significant alteration in the memory of rats when compared to the control group. On the other hand, rats in the SF-I group showed a significant ($p<0.01$) improvement in the spatial memory compared to the SF-II group.

4. Hematological and biochemical parameters

For erythrogram, there were no statistically significant variations between experimental groups. However, in the leucogram, there was significant leucopenia accompanied by absolute lymphopenia in SF-II in comparison with the control and SF-I groups.

For ALT and AST activities, there was a significant reduction in their activities in the SF-II group compared to the control and SF-I groups (Table 3).

Table 3: Effect of single oral treatment of *S. officinalis* terpenoids-rich fraction on different hematological and biochemical parameters.

	Groups / Parameters	Control	SF-I	SF-II
Hematology	Erythrocyte (×10^6/µL)	7.25 ± 0.23	7.11 ± 0.31	7.03 ± 0.02
	PCV %	42.67± 0.88	42.67± 1.45	43.00 ±0.58
	Hb (g/dl)	12.00 ± 0.12	11.60 ± 0.06	11.67 ± 0.23
	Leucocyte (×10^3/µL)	14.27 ± 0.07 ^{a}	14.47 ± 0.49 ^{a}	10.88 ± 0.52 ^{b}
	Lymphocyte (×10^3/µL)	12.07 ± 0.15 ^{a}	11.37 ± 0.30 ^{a}	8.69 ± 0.60 ^{b}
	Neutrophil (×10^3/µL)	1.09 ± 0.10	1.70 ± 0.29	1.01 ± 0.13
	Eosinophil (×10^3/µL)	0.43 ± 0.08	0.53 ± 0.04	0.47 ± 0.02
	Monocytes (×10^3/µL)	0.67 ± 0.05	0.87 ± 0.03	0.71 ± 0.12
Biochemistry	ALT (U/L)	23.40 ± 0.40 ^{a}	21.70 ± 1.03 ^{a}	14.28 ± 0.26 ^{b}
	AST (U/L)	57.12 ± 0.32 ^{a}	61.66 ± 0.73 ^{a}	43.68 ± 1.77 ^{b}
	Urea (mg/dl)	55.30 ± 1.39	56.88 ± 1.05	56.70 ± 1.06
	Creatinine (mg/dl)	0.63 ± 0.10	0.49 ± 0.02	0.57 ± 0.10

Data are expressed as means ± SE (n=5) with dissimilar superscript letters in each row (significantly differing at $P < 0.05$). Control: control negative group; SF-I: Salvia fraction 2 g/kg b.w; SF-II: Salvia fraction 3 g/kg b.w; PCV: Packed cell volume; Hb: Hemoglobin; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

5. Histopathological results

The histopathological pictures of hepatic and renal tissues were illustrated in Figure 5. Hepatic tissue sections stained with H&E from normal rats showed a normal central vein and radiating hepatic cords (Figure 5, A).

The stained hepatic sections of most rats in the SF-I group (2 g/kg b.w) revealed no detected microscopic lesions (Figure 5, B). While most rats in the SF-II group received 3 g/kg b.w showed mononuclear cell infiltration within the portal triads, vasculitis, perivascular edema in association with activation of kupffer cells and dilatation of hepatic sinusoids, vaculation of hepatocytes, and few intralobular infiltrations of lymphocytes. There was proliferation of hepatic fibrous connective tissue. In some instances, mononuclear cells distributed in a focal manner as nodules with congestion (Figure 5, C). As well as renal tissues from normal rats (Control), showed no necrobiotic variations with normal histological features of the kidney (glomeruli and renal tubules) (Figure 5, D). The stained sections of salvia fraction-treated rats' kidneys in the SF-I group (2 g/kg b.w) revealed mild congestion (Figure 5, E). While rats received 3 g/kg b.w (SF-II) presented necrosis of some proximal convoluted tubules, thickening of basement membrane, and mild fibrosis. Some renal epithelial cells underwent regeneration in the form of cellular mass without lumen (Figure 5, F).

Figure 1: The chromatogram obtained from LC-MS analysis of *S. officinalis* terpenoids rich fraction; the pie chart indicates the relative percentage of identified compounds according to their classes.

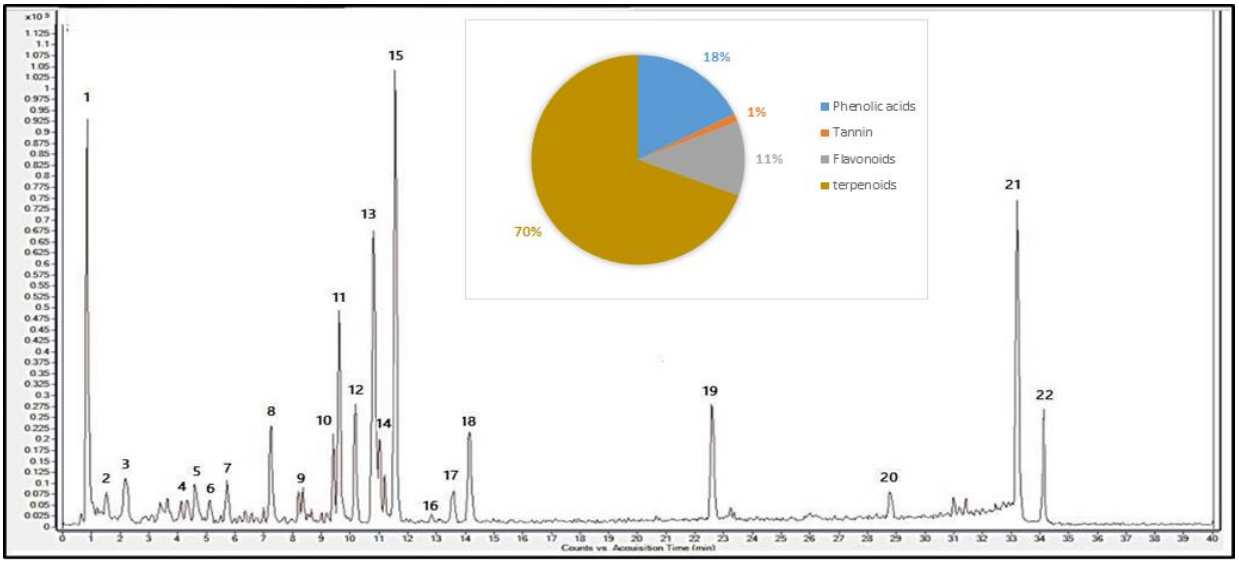
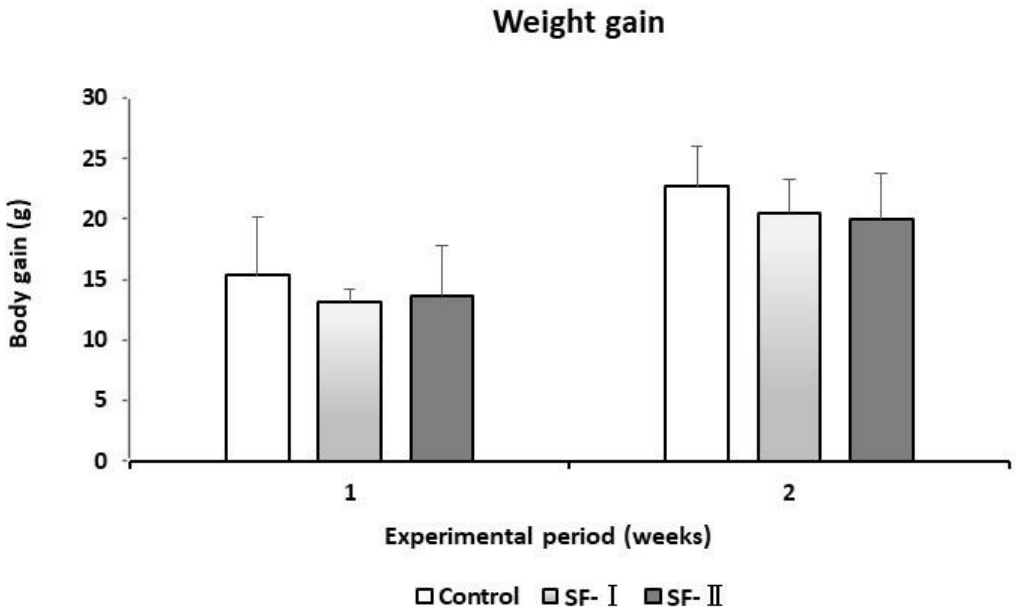


Figure 2: The weekly weight gains of rats after single oral treatment of *S. officinalis* terpenoids rich fraction.



Control: control negative group.
SF-I: Salvia fraction 2 g/kg b.w.
SF-II: Salvia fraction 3 g/kg b.w.

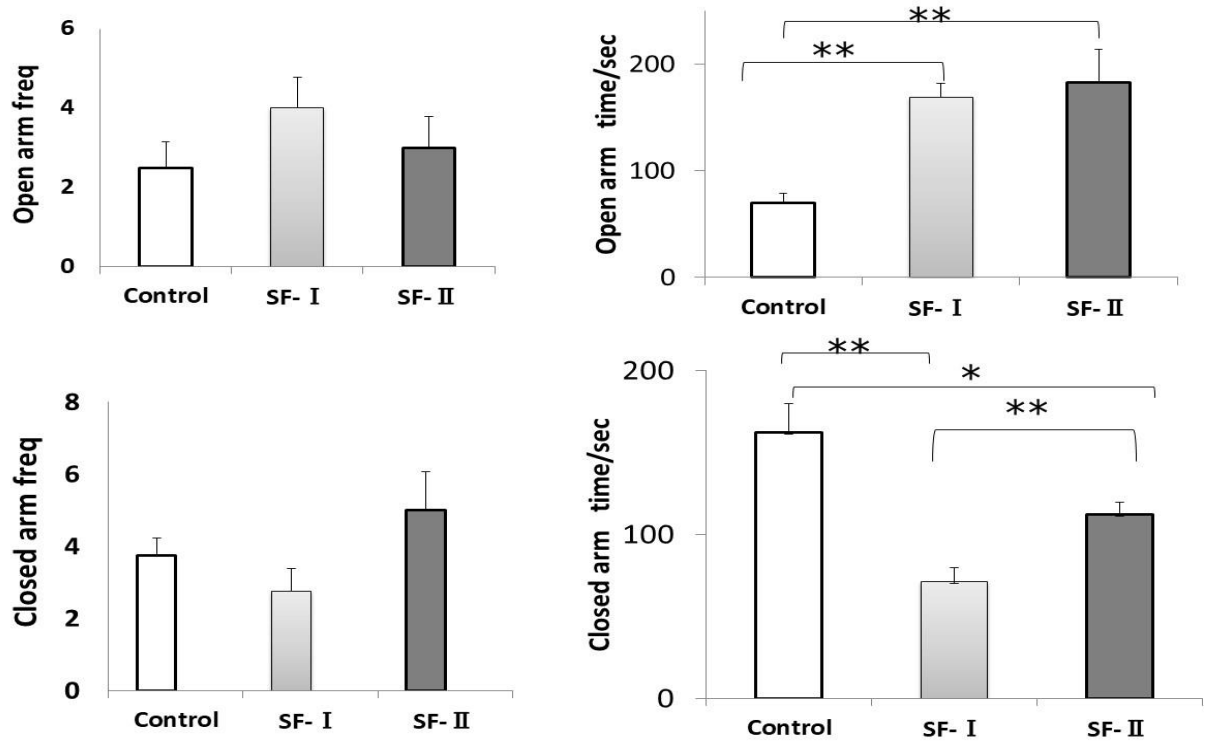


Figure 3: Effect of single oral treatment of *S. officinalis* terpenoids rich fraction on anxiety-like behavior in elevated plus maze.
Control: control group; SF-I: Salvia fraction 2 g/kg b.w; SF-II: Salvia fraction 3 g/kg b.w.

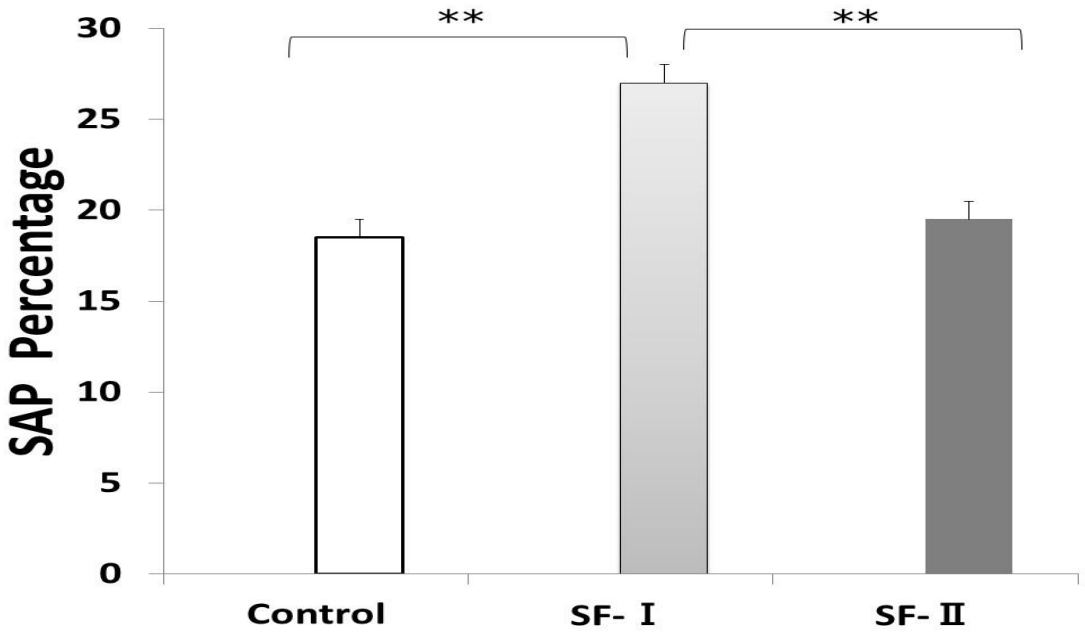


Figure 4: Effect of single oral treatment of *S. officinalis* terpenoids rich fraction on working memory of rats in y-maze.

C: control group; SF-I: Salvia fraction 2 g/kg b.w; SF-II: Salvia fraction 3 g/kg b.w.

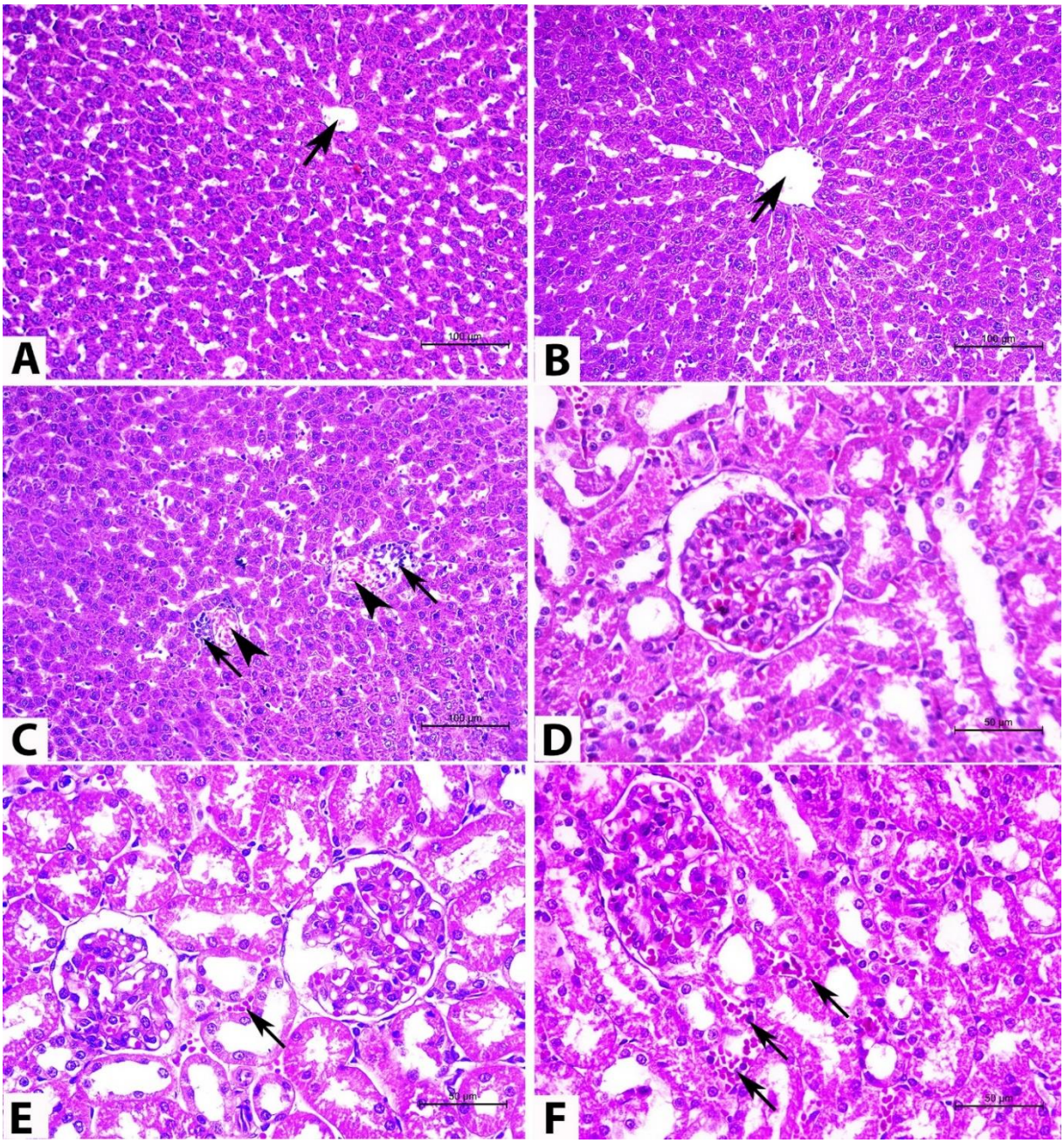


Figure 5: Photomicrograph of representative tissue samples from rats' livers and kidneys**. (A**) Normal rats presented a normal central vein **(arrow)** and radiating hepatic cords. (**B**) Livers in the **SF-I** group (2 g/kg b.w) revealed no detected microscopic lesions. **(C)** Rats in the SF-II group (3 g/kg b.w) showed mononuclear cell infiltration within the portal triads **(arrow)** and congestion **(head arrow). (D)** Renal tissues from normal negative control rats revealed normal renal histology characterized by glomeruli and renal tubules. **(E)** Kidneys of rats in the **SF-I** group (2 g/kg b.w) revealed mild congestion **(arrow)**. **(F)** Rats in the **SF-II** group (3g/kg b.w) presented congestion **(arrow)** and thickening of the basement membrane **(H&E, x200, x400)**.

DISCUSSION

In recent years, the use of medicinal plants in different aspects has been given major attention. *Salvia officinalis* L., a widely spread member of the Lamiaceae family, has been utilized as an herbal medicine against different diseases. The main concern of this paper is to evaluate the safety of *S. officinalis* terpenoids-rich fraction after single oral administration at doses of 2 and 3 g/kg b.w.

Assessment the safety of herbal extracts includes determining the changes in body weight and weight gain. In our study, the administration of *S. officinalis* terpenoids-rich fraction up to 3 g/kg b.w produced no prominent variation in weight gain when compared with the non-treated control group, which emphasizes the safety of this fraction. Our result agreed with a recent study by Rhaimi *et al.* (2023) investigating the toxicity of *S. officinalis* essential oil administered at levels of 1, 2, and 3 g/kg b.w as a single dose and found that there was no meaningful variation in body weight through the 14-day experimental period.

The obtained results indicated that *S. officinalis* (2 g/kg b.w) induced no prominent effect on locomotor behavior of rats in the open field maze; on the other hand, it increased with the highest dose of Salvia fraction, but this increment didn't affect anxiety-like behavior evaluated with the same maze.

In addition, both doses of *S. officinalis* had anxiolytic effects on rats in the EPM. Moreover, *S. officinalis* improved the memory of rats only at 2 g/kg b.w dose. In the early study, Choukairi *et al.* (2019), they found that locomotion of rats did not alter by intraperitoneal (i.p.) injection of 25µg/ml of *S. officinalis*. Additionally, Mora et al. (2006) reported that 3, 12, 12.5, 25, and 50 mg/kg b.w of *S. officinalis* extract diminished the locomotion of rats, and only at the dose of 12.5 mg/kg b.w their anxiety was reduced in the EPM. Moreover, Rhaimi *et al.* (2023) observed that *S. officinalis* essential oil (1 g/kg b.w) had anxiolytic and antidepressant effects and enhanced memory in rats.

Oxidative stress is usually accompanied with behavioral disorders (Lamtai *et al.*, 2020). Therefore, the antioxidant efficacy of *S. officinalis* may be one of the possible explanations of the observed effect in our study. The improvement of rats` memory in our study may be due to the CNS cholinergic receptor binding activity of *S. officinalis* (Wake et al., 2000). Ayoub et al. (2022) explained the memory enhancement induced by *S. officinalis* to ameliorate of oxidative stress markers and acetylcholinesterase activity in rats. In addition, Linalool is one of the main components of *S. officinalis* essential oil extract (Makino *et al.,* 1996) that has a sedative function at the CNS (Jirovetz *et al.,* 1991), which may be the main cause of the observed anxiolytic effect (Mora *et al.,* 2006).

However, in the current study, the sedative effect may be by virtue of the flavonoids and phenolic acids in the used fraction, as previously mentioned by Hasanein *et al.* (2015), who showed that the hydroalcoholic extract of *S. officinalis* reduced various behaviors concomitant with morphine withdrawal in rats by producing non-selective sedative actions as a result of the antioxidant efficacy of polyphenols and flavonoids in their extract.

Our attention was focused not only on evaluating the effects of *S. officinalis* fraction on behavior patterns and body weight but also on general health status.

In toxicity studies, the estimation of hematological parameters thought to be the most vulnerable to toxins or their metabolites. Therefore, hematological alterations are considered a good predictive value for toxicity in humans or animals (Arika *et al.,* 2016).

The hematological data in the present study illustrated that administration of *S. officinalis* terpenoids-rich fraction up to 2 g/kg b.w had no negative impact on measured parameters compared to the control rats, proving the safety of the fraction.

For the rats receiving fraction at 3 g/kg b.w dose, there was leucopenia due to absolute lymphopenia. Based on previous reports, the leukocyte acts as the first line of defense against microorganisms and plays a vital role in innate immunity, corresponding with tissue damage and inflammatory responses (Chen *et al.,* 2018; Marshall *et al.,* 2018).

Leucopenia due to lymphopenia in those rats may be a result of lymphocytic infiltration in hepatic tissue, as shown in histopathological examination, leading to a reduction of circulating lymphocytes. Ramos *et al.* (2015) evaluated D-Limonene-induced hepatotoxicity, a monoterpene found in different plants, using immunohistochemistry and found that influx of T (CD3+) and cytotoxic (CD8+) lymphocytes in liver tissue. This influx with activation of Kupffer cells may be an indication of an immunological response triggered by d-limonene reactive metabolites.

Therefore, the changes we observed in hepatic and renal tissues in our study may be a consequence of the direct impact of fraction components and their reactive metabolites on these tissues.

The renal and hepatic functions become one of the most important toxicological assessments of plant extracts because they are responsible for several functions to ensure an organism's survival (Al-Afifi *et al.,* 2018). In the present study, regardless of the used dose, administration of *S. officinalis* terpenoids-rich fraction induced no significant change in levels of urea and creatinine. Our finding is in line with the previous work of Bouteldja *et al.,* 2023, who stated that the oral administration of *S. officinalis* ethanolic extract as a 2 g/kg b.w single dose produced insignificant variation in urea and creatinine levels after two weeks of administration, confirming the non-toxicity of the plant.

Also in our study, serum ALT and AST activities were reduced in rats receiving the highest dose of Salvia fraction as compared to the control group. This alteration wouldn't be significant, as the reduction of serum aminotransferase levels below the lower limit of normal has no clinical importance (Pratt, 2010). When serum transaminases elevate at least three folds above the normal limit, it is considered evidence for a substance-induced hepatic injury (Chang and Schiano, 2007).

In the study of Muthuviveganandavel *et al.* (2008), the toxicity of carbendazim in rats reduced serum transaminase activities with no prominent histopathological alteration in hepatic tissue.

CONCLUSION

The single oral administration of *Salvia officinalis* terpenoids rich fraction in Wistar rats as 2 g/kg b.w dose had clear anxiolytic with memory-enhancing effects and did not induce mortality, toxicity signs, or significant variation in weight gain, hemato-biochemical parameters, or histopathological features. While the higher dose of *Salvia officinalis* terpenoids rich fraction (3 g/kg b.w) increased locomotor behavior, had anxiolytic effect, and induced mild alterations in hematological parameters and histopathological features.

Therefore, *Salvia officinalis* terpenoids-rich fraction appears to be safe at higher doses.

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Conflicts of Interest

No conflict of interest is declared by the authors.

REFERENCES

Al-Afifi, N.A.; Alabsi, A.M.; Bakri, M.M. and Ramanathan, A. (2018): Acute and sub-acute oral toxicity of Dracaena cinnabari resin methanol extract in rats. BMC Complement. Altern. Med. 18, 1–14. https://doi.org/10.1186/s12906-018-2110-3

Al Musayeib, N.M.; Mothana, R.A.; Mohamed, G.A.; Ibrahim, S.R. and Maes, L. (2014). Hypoestenonols A and B, new fusicoccane diterpenes from Hypoestes forskalei. Phytochemistry Letters, 10, 23-27. https://doi.org/10.1016/j.phytol.2014.06.020

Arika, W.; Nyamai, D.; Musila, M.; Ngugi, M. and Njagi, E. (2016): Hematological markers of in vivo toxicity. J. Hematol. Thromboembolic. Dis. 4, 1–7. DOI:10.4172/2329-8790.1000236

Ayoub, I.M.; George, M.Y.; Menze, E.T.; Mahmoud, M.; Botros, M.; Essam, M.; Ashmawy, I.; Shendi, P.; Hany, A.; Galal, M.; Ayman, M. and Labib, R.M. (2022): Insights into the neuroprotective effects of Salvia officinalis L. and Salvia microphylla Kunth in the memory impairment rat model. Food Funct. 13(4): 2253–2268. https://doi.org/10.1039/D1FO02988F

Bancroft, J.D. and Layton, C. (2013): The hematoxylin and eosin. In: Suvarna SK, Layton C, Bancroft JD (eds) Theory practice of histological techniques, 7th edn. Churchill Livingstone of El Sevier, Philadelphia, pp 179–220. http://dx.doi.org/10.1016/b978-0-7020-4226-3.00010-x.

Boros, B.; Farkas, A.; Jakabová, S.; Bacskay, I.; Kilár, F. and Felinger, A. (2010): LC-MS quantitative determination of atropine and scopolamine in the floral nectar of Datura species. Chromatographia, 71, 43-49. https://doi.org/10.1365/s10337-010-1524-y

Bouteldja, R.; Doucene, R.; Aggad, H.; Abdi, F.Z.; Belkhodja, H.; Belal, A.; Abdall, M. and Zidane, K. (2023): Phytochemical Screening, Acute Toxicity and Antidiabetic Activity of Ethanolic Extract of Salvia officinalis L. in Wistar Rat. Agric. conspec. sci. 88(4), 351-357.

Bowers, L.D. and Wong, E.T. (1980): Kinetic serum creatinine assays. II. A critical evaluation and review. Clinical chemistry 26(5), 555-561. https://doi.org/10.1093/clinchem/26.5.555

Brown, R.E.; Corey, S.C. and Moore, A.K. (1999): Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. Behav. Genet. 29, 263-271. https://doi.org/10.1023/A:1021694307672

Chang, C.Y. and Schiano, T.D. (2007): Drug hepatotoxicity. Aliment. Pharmacol. Ther. 25(10), 1135-1151. https://doi.org/10.1111/j.1365-2036.2007.03307.x

Chen, L.; Deng, H.; Cui, H.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X. and Zhao, L. (2018): Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9, 7204–7218. DOI: 10.18632/oncotarget.23208

Choleris, E.; Thomas, A.W.; Kavaliers, M. and Prato, F.S. (2001): A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field.

Neurosci. Biobehav. Rev. 25(3), 235-260. https://doi.org/10.1016/S0149-7634(01)00011-2

Choukairi, Z.; Hazzaz, T.; Lkhider, M.; Ferrandez, J.M. and Fechtali, T. (2019): Effect of Salvia Officinalis L. and Rosmarinus Officinalis L. leaves extracts on anxiety and neural activity. Bioinformation 15(3), 172. DOI: 10.6026/97320630015172

Drabkin, D.L. and Austin, J.H. (1932): Spectrophotometric studies: I. Spectrophotometric constants for common hemoglobin derivatives in human, dog, and rabbit blood. J. Biol. Chem. 98(2), 719-733. https://doi.org/10.1016/S0021-9258(18)76122-X

Feldman, B.F.; Zinkl, J.G. and Jain, V.C. (2000): In: Schalm's Veterinary hematology. 5th Ed. Lippincott Williams and Wilkins, Canada, pp. 1145-1146.

Gould, T.D.; Dao, D.T. and Kovacsics, C.E. (2009): The open field test. In; Mood and anxiety related phenotypes in mice: characterization using behavioral tests. Gould, T.D., editor. Humana Press, Totowa, NJ, USA, pp.1-20. https://doi.org/10.1007/978-1-60761-303-9

Hasanein, P.; Teimuri Far, M. and Emamjomeh, A. (2015): Salvia officinalis L. attenuates morphine analgesic tolerance and dependence in rats: possible analgesic and sedative mechanisms. Am. J. Drug. Alcohol. Abuse. 41(5), 405-413. https://doi.org/10.3109/00952990.2015.1062893

Jain, N.C. (1993): In: Essentials of Veterinary Haematology. Lea and Febiger, Philadelphia (Pa), USA, pp. 76–250.

Jirovetz, L.; Jager, W.; Buchbauer, G.; Nikiforov, A. and Reverdino, V. (1991): Investigations of animal blood samples after fragrance drug inhalation by gas chromatography/mass spectrometry with chemical and selected ion monitoring. Biol. Mass. Spectrom. 20 (12), 801–803. https://doi.org/10.1002/bms.1200201210

Kalueff, A. and Tuohimaa, P. (2004): Experimental modeling of anxiety and depression. Acta. Neurobiol. Exp. 64(4), 439-448.

Komada, M.; Takao, K. and Miyakawa, T. (2008): Elevated plus maze for mice. J. Vis. Exp. (22), e1088. DOI: 10.3791/1088.

Lamtai, M.; Zghari, O.; Ouakki, S.; Marmouzi, I.; Mesfioui, A.; El Hessni, A. and Ouichou, A. (2020): Chronic copper exposure leads to hippocampus oxidative stress and impaired learning and memory in male and female rats. Toxicol. Res. 36(4), 359–366. https://doi.org/10.1007/s43188-020-00043-4

Li, L.; Wei, S.; Zhu, T.; Xue, G.; Xu, D.; Wang, W.; Wang, X.; Luo, J. and Kong, L. (2019): Anti-inflammatory norabietane terpenoids from the leaves of Salvia officinalis L. J. Funct. Foods. 54, 154-163. https://doi.org/10.1016/j.jff.2019.01.020

Llurba-Montesino, N. and Schmidt, T.J. (2018): Salvia species as sources of natural products with antiprotozoal activity. Int. J. Mol. Sci. 19(1), 264. https://doi.org/10.3390/ijms19010264

Makino, T.; Ohno, T. and Iwabuchi, H. (1996): Aroma components of pinneaple sage (Salvia elegans Vahl). Foods Food Ingred. J. Jpn. 169, 121–124.

Marshall, J.S.; Warrington, R.; Watson, W. and Kim, H.L. (2018): An introduction to immunology and immunopathology. Allergy. Asthma. Clin. Immunol. 14, 1–10. https://doi.org/10.1186/s13223-018-0278-1

Mora, S.; Millán, R.; Lungenstrass, H.; Díaz-Véliz, G.; Morán, J.A.; Herrera-Ruiz, M. and Tortoriello, J. (2006): The hydroalcoholic extract of Salvia

elegans induces anxiolytic-and antidepressant-like effects in rats. J. Ethnopharmacol. 106(1), 76-81. https://doi.org/10.1016/j.jep.2005.12.004

Muthuviveganandavel, V.; Muthuraman, P.; Muthu, S. and Srikumar, K. (2008): Toxic effects of carbendazim at low dose levels in male rats. J. Toxicol. Sci. 33(1), 25-30. https://doi.org/10.2131/jts.33.25

Nasri, S.; Roghani, M.; Baluchnejadmojarad, T.; Balvardi, M. and Rabani, T. (2012): Chronic cyanidin-3-glucoside administration improves short-term spatial recognition memory but not passive avoidance learning and memory in streptozotocin-diabetic rats. Phytother. Res. 26(8), 1205-1210. https://doi.org/10.1002/ptr.3702

OECD, O. (2001): 423-Guidelines for the Testing of Chemicals Acute Oral Toxicity-Fixed Dose Procedure. Animals. 1–14.

Patton, C.J. and Crouch, S.R. (1977): Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem. 49(3), 464-469. https://doi.org/10.1021/ac50011a034

Pratt, D.S. (2010): Liver chemistry and function tests. In: Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 9th Ed. Saunders Elsevier, Philadelphia (Pa): pp. 1243–1246.

Ramos, C.A.F.; Sá, R.D.C.D.S.; Alves, M.F.; Benedito, R.B.; de Sousa, D.P.; Diniz, M. D.F.F.M.; Araújo, M.S.T. and de Almeida, R.N. (2015): Histopathological and biochemical assessment of d-limonene-induced liver injury in rats. Toxicol. Rep. 2, 482-488. https://doi.org/10.1016/j.toxrep.2015.01.001

Rasoulijazi, H.; Joghataei, M.T.; Noubakht, M. and Roughani, M. (2007): The beneficial effect of (-)-epigallocatechin-3-gallate in an experimental model of Alzheimer's disease in rat: A behavioral analysis. Iran. Biomed. J. 11, 237–243.

Reitman, S. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28(1), 56-63. https://doi.org/10.1093/ajcp/28.1.56

Rhaimi, S.; Brikat, S.; Lamtai, M. and Ouhssine, M. (2023): Acute oral toxicity and neurobehavioral effects of Salvia officinalis essential oil in female Wistar rats. Adv. Anim. Vet. Sci.11(4), 654-62. https://doi.org/10.17582/journal.aavs/2023/11.4.654.662

Salamatin, A.A.; Khaliullina, A.S. and Khaziev, R.S. (2020): Extraction of aromatic abietane terpenoids from Salvia officinalis leaves by petroleum ether: Data resolution analysis. Ind. Crop. Prod. 143, 111909. https://doi.org/10.1016/j.indcrop.2019.111909

Shivaraj, Y.; Kaveri, K.; Asiya Nuzhat, F. and Rajesh, R. (2021): Acute oral toxicity study of "Polyherbal formulation (Rosmarinus officinals, Ashwaghandha and Amla) in Wistar rats". Journal of Entomology and Zoology Studies 9(4), 16-27. https://doi.org/10.22271/j.ento.2021.v9.i4a.8752

Thrall, M.A.; Baker, D.C. and Lassen, E. (2004): In: Veterinary Hematology and Clinical Chemistry. Lippincott Williams and Wilkins, Philadelphia (Pa), USA.

Vuković, G.; Bursić, V.; Stojanović, T.; Petrović, A.; Konstantinović, B.; Puvača, N. and Marinković, D. (2020): Determination of atropine and scopolamine in popcorn by the LC-MS/MS. In 26th International Symposium on Analytical and Environmental Problems (pp. 23-24).

Wake, G.; Court, J.; Pickering, A.; Lewis, R.; Wilkins, R. and Perry, E. (2000): CNS acetylcholine receptor activity in European medicinal plants

traditionally used to improve failing memory. J. Ethnopharmacol. 69, 105–114. https://doi.org/10.1016/S0378-8741(99)00113-0

Wall, P.M.; Blanchard, R.J.; Markham, C.; Yang, M. and Blanchard, D.C. (2004): Infralimbic D1 receptor agonist effects on spontaneous novelty exploration and anxiety-like defensive responding in CD-1 mice. Behav. Brain. Res., 152(1), 67-79. https://doi.org/10.1016/j.bbr.2003.09.035

دراسة الامان الحاد للجزء الغني ب داتربينويدات من نبات المريمية فى الفئران

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أصبحت نباتات السالفية أكثر شعبية واستخدام بسبب إمكاناتها المعززة للصحة ومضادات الأكسدة. تهدف الدراسة الحالية إلى التحقق من حدود الأمان للجزء الغني ب داتربينويدات من نبات المريمية فى الفئران. تم تقسيم ثلاثين من إناث الفئران بالتساوي إلى ثلاث مجموعات. المجموعة الأولى غير معالجة (كنترول). تلقت المجموعتان الثانية والثالثة الجزء الغني ب داتربينويدات من المريمية كجرعة فموية واحدة ٢ و ٣ جم/كجم من وزن الجسم على التوالي. خلال فترة التجربة، تمت ملاحظة أي علامات سريرية غير طبيعية على الفئران ، وتم تسجيل معدلات الوفيات بالإضافة إلى وزن الجسم أسبوعيًا. وفي نهاية التجربة تم إجراء اختبارات السلوك وجمع عينات الدم لإجراء فحوصات الدم والكيمياء الحيوية. تم جمع الأعضاء (الكبد والكلى) من أجل للفحص الباثولوجى. لم تكن هناك اختلافات غير طبيعية في الاعراض السريرية أو الوزن في كلتا الجرعتين. كان للجزء الغني ب داتربينويدات من المريمية في كلتا الجرعتين تأثير مزيل القلق مميز؛ ومع ذلك، فقد تحسنت الذاكرة العاملة فقط عند جرعة ٢ جم/كجم من وزن الجسم. حدثت زيادة في النشاط الحركي في الفئران التي تلقت ٣ جم/كجم من وزن الجسم. بالنسبة لفحوصات الدم والكيمياء الحيوية السريرية، كان هناك نقص بسيط في خلايا الدم البيضاء مصحوب بنقص مطلق لخلايا ليمفوسيت وانخفاض في نشاط انزيمات الكبد في الفئران التي تلقت ٣ جم/كجم من وزن الجسم ، إلى جانب عدم وجود تغير كبير في اليوريا والكرياتينين في كلا المجموعتين المعالجتين. لذلك، يبدو أن الجزء الغني الغني ب داتربينويدات من نبات المريمية ليس له آثار سمية ضارة، باستثناء التغيرات البسيطة في خلايا الدم البيضاء، وأنزيمات الكبد، والأنسجة الكبدية والكلوية المحدثة في الفئران المعالجة بـ ٣ جم/كجم من وزن الجسم.

الكلمات االمفتاحية: نبات المريمية، داتربينويدات، الامان، الفئران.