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Assessment of the acute and subacute toxicity of Algerian *Hyoseris radiata* L. in the Wistar albino rats model

Evaluación de la toxicidad aguda y subaguda de *Hyoseris radiata* L. argelina en el modelo de ratas albinas Wistar

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ABSTRACT

Wild chicory, or *Hyoseris radiata* L., is indigenous to the Mediterranean region, is a plant used in traditional medicine as a diuretic, blood depurative, and against kidney stones. The present study aimed to assess for the first time the acute and subacute toxicity, to quantify the total amount of polyphenols and flavonoids, and to assess the antioxidant activity of *H. radiata* collected from Setif, Algeria. The overall amount of flavonoids and polyphenols was quantified spectrophotometrically. The antioxidant activity of the extract was evaluated according to two methods, DPPH and FRAP. The acute toxicity of *H. radiata* was carried out according to the OECD guideline 423 to determine the median lethal dose LD_{50} and the subacute toxicity was evaluated according to OECD guideline 407 to assess the possible pathological effects of the extract administered for 28 days by oral route. The results show that the total amount of polyphenols and flavonoids was 132.53 ± 2 µg of GAE·1 mg^{-1} and 96.11 ± 3.65 µg of QE·1 mg^{-1} of extract, respectively. The extract shows a good antioxidant potential in both tests. The administered dose (2 g·kg^{-1} of BW) didn't produce any changes in general behaviors or mortality, so the LD_{50} is greater than 2 g·kg^{-1} of BW. Moreover, the daily administration of the extract with 2 doses, 100 mg·kg^{-1} and 200 mg·kg^{-1} didn't cause any changes in body weight, behavior test, hematological parameters, and organ relative weight. A significant decrease in triglyceride was recorded in both concentrations. Based on the present findings, the extract of *H. radiata* has no significant toxicity. These findings offer valuable information about the toxicity profile of the traditional medicine plant *Hyoseris radiata* L.

Keywords: *Hyoseris radiata*; acute toxicity; subacute toxicity; antioxidant activity

RESUMEN

La achicoria silvestre, o *Hyoseris radiata* L., es autóctona de la región mediterránea y se utiliza en la medicina tradicional como diurético, depurativo de la sangre y contra los cálculos renales. El presente estudio tuvo como objetivo evaluar por primera vez la toxicidad aguda y subaguda, cuantificar la cantidad total de polifenoles y flavonoides, y evaluar la actividad antioxidante de *H. radiata* recolectada en Setif, Argelia. La cantidad total de flavonoides y polifenoles se cuantificó espectrofotométricamente. La actividad antioxidante del extracto se evaluó mediante dos métodos: DPPH y FRAP. La toxicidad aguda de *H. radiata* se realizó de acuerdo con la directriz 423 de la OCDE para determinar la DL_{50} letal media, y la toxicidad subaguda se evaluó de acuerdo con la directriz 407 de la OCDE para evaluar los posibles efectos patológicos del extracto administrado durante 28 días por vía oral. Los resultados muestran que la cantidad total de polifenoles y flavonoides fue de 132,53 ± 2 µg de GAE·1 mg^{-1} y 96,11 ± 3,65 µg de QE·1 mg^{-1} mg de extracto respectivamente. El extracto muestra un buen potencial antioxidante en ambas pruebas. La dosis administrada (2 g·kg^{-1} de peso corporal) no produjo cambios en los comportamientos generales ni en la mortalidad, por lo que la DL_{50} es mayor a 2 g·kg^{-1} de peso corporal. Además, la administración diaria del extracto en 2 dosis de 100 mg·kg^{-1} y 200 mg·kg^{-1} no provocó cambios en el peso corporal, la prueba de comportamiento, los parámetros hematológicos y el peso relativo de los órganos. Se registró una disminución significativa de los triglicéridos en ambas concentraciones. Según los hallazgos actuales, el extracto de *H. radiata* no tiene una toxicidad significativa. Estos hallazgos proporcionan información importante sobre el perfil de toxicidad de la planta de medicina tradicional *Hyoseris radiata* L.

Palabras clave: *Hyoseris radiata*; toxicidad aguda; toxicidad subaguda; actividad antioxidante

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INTRODUCTION

For a long time, across various cultures and civilizations, herbal medicinal plants have been utilized as treatments for both preventing and curing a variety of illnesses because of their potent therapeutic benefits and low prices. People used to eat them because they believed that natural products were safe and had no negative effects [1].

However, while herbal medicines are often considered safe due to their natural origins, they are not devoid of risks because safe and natural are not synonyms [2]. Many plants contain bioactive compounds, some of which are very complex and can exhibit toxicity if consumed inappropriately. Nevertheless, in general, herbal remedies are utilized to treat certain illnesses without any scientific understanding or proof of their harmful effects. And many species have not been toxicologically validated; that's why there is a need to monitor their safety [3, 4].

The combined techniques of acute and subacute toxicity testing are used to assess the toxicity or adverse effects of chemicals and several herbal remedies, as well as to examine their mode of action [5] . Knowledge produced by these tests is used in the detection and risk administration of chemicals [6]. Acute toxicity is investigated with a single dose performed to look into the signs and severity of toxicity that affect laboratory animals [7] . Following 28 days (d) of repeated chemical administration, the subacute toxicity test is performed to confirm the harmed desired organ or tissue of the experimental animals [8].

Hyoseris radiata L.*,* a wild plant known as wild chicory, belongs to the Asteraceae family and is belong to the Mediterranean region (Italy, Malta, France, Spain, Greece, Algeria, ex–Yugoslavia, Morocco, Tunisia, the Canary Islands, and Turkey). It grows between the dry wall's rocks, on roadsides, and uncultivated fields at a height up to 1000 m [9].

This wild plant has been used in the preparation of traditional food and to cure a variety of diseases in traditional Medicine [10]. Popular medicine in Italy suggests drinking an infusion of its leaves as a blood depurative, diuretic, or against kidney stones as a litholithic. The leaves are also frequently boiled as an intestinal depurative and to prevent severe constipation. Additionally, the raw leaves can be fed to sheep and rabbits [11]. Despite its widespread usage in traditional medicine and cooking, *H. radiata* remains insufficiently explored and studied to date, and an assessment of this herb's safety is required. This study's objective is to assess for the first time the acute and subacute toxicity of *Hyoseris radiate* L. and to determine its total content in polyphenols and flavonoids, and to test its antioxidant activity.

MATERIALS AND METHODS

Plant material

H. radiata leaves were collected in January 2023 from El Hamma, Setif, Algeria, and identified by Professor Bekdouche at the University of Batna 2. Then they were dried in a dry place away from light and ground into powder (Retsch, SK 100, Germany).

Preparation of the extract

By maceration of 250 g of the powder in 2.5 L of methanol(80%) for 48 h with agitation; after that, the mixture was vacuum filtered using a vacuum pump (Sparmax, TC-501V, Taiwan) with a filtration apparatus (Millipore, Filtration apparatus, USA) and evaporated at 40°C(Büchi, Rotavapor R-3, Switzerland), and the extract yield was calculated [12].

Total polyphenols

The Folin–Ciocalteu method was used to assess the total amount of polyphenols [13]. 20 µL of the extract was mixed with 100 µL of Folin–Ciocalteu reagent, after 4 min, 80 µL of sodium carbonate was added. The absorbance was measured at 765 nm (PerkinElmer, EnSpire multimode plate reader, USA) following a 2 h incubation period at room temperature. The calibration curve used to determine the quantity of total polyphenols in the extract was established using Gallic acid (25–200 $\mu g \cdot mL^{-1}$) as the standard.

Total flavonoids

The aluminum chloride ($AlCl_3$) procedure described by Turkoglu *et al.* [14] was used to determine the total flavonoid content of the hydro–methanolic extract of *H. radiata* (HMEHR). 100 µL of the extract was combined with 100 µL of a methanolic solution of $AlCl_3$, and the resulting mixture was rapidly agitated. The mixture's absorbance at 430 nm was measured (PerkinElmer, EnSpire multimode plate reader, USA) following a 10 min incubation period at room temperature. The calibration curve for estimating the quantity of flavonoids was established using quercetin (25–200 $\mu g \cdot mL^{-1}$.

Assessment of *in vitro* antioxidant capacity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging ability

The DPPH solution (160 µL) was mixed with 40 µL of the extract at different concentrations. The absorbance was measured at 517 nm after 30 min of incubation at room temperature against a blank (solution of DPPH/methanol) [15], and Ascorbic acid was used as a reference antioxidant. The percentage of antioxidant activity was determined according to the following equation:

$$I\,\% = \frac{absorbance\ of\ the\ blank - absorbance\ of\ the\ sample}{absorbance\ of\ the\ blank} \times 100$$

Reducing ability

The reducing potential of the extract was evaluated using the method described by Oyaizu [16]. 50 µL of $K_3Fe(CN)_6$ (1%) and 40 µL of phosphate buffer (pH 6.6) were added to 10 µL of our extract at different concentrations. After 20 min of incubation at 50°C (Binder ED 53, Germany), 50 µL of TCA (10%), 40 µL of distilled water, and 10 µL of $FeCl_3$ (0,1%) were added. Then, the absorbance was read at 700 nm. Ascorbic acid was employed as a positive control, and the results were expressed in A0.5 (Absorbance of 0.5).

Experimental animals

Experiments were performed using nulliparous and non–pregnant female Wistar albino rats (*Rattus norvegicus*) from the Pasteur Institute of Algeria, aged 9 to 11 weeks and weighing

between 210 and 250 g (OHAUS, Navigator™ NV422, USA). Animals were housed in standard cages and acclimated to laboratory conditions for one week. They were kept under standard regular conditions at humidity between 30–50% and a temperature of 22 ± 3°C with a 12 h light/12 h dark cycle, provided with standard animal feed and given free access to water for drinking.

Acute toxicity

This toxicity was evaluated following the OECD (Organization for Economic Co–operation and Development) guideline 423 [7]. Animals were divided into 2 groups (test and control), each group contained 6 rats. After fasting overnight, each animal from the test group received by gavage one single dose of HMEHR 2000 g·kg^{-1} body weight dissolved in distilled water (6 mL·kg^{-1}) with 2% of dimethylsulfoxide (DMSO), and each rat from the control group received distilled water with 2% of DMSO. Following a 3 h fasting period, animals were individually monitored during the first 30 min, then at regular intervals for the first 24 h, and then once daily over 14 d.

Observation should also include mortality, eyes and mucous membranes, skin and fur changes, behavior pattern, sleep, lethargy, diarrhea, salivation, and coma. During the study period, the weights of animals were recorded at the beginning and then once a week. At the end of the test, surviving animals were weighed and humanely euthanized.

Subacute toxicity

The test was conducted following the OECD's recommendations (Guideline–n°407) [8]. Animals were randomly divided into 3 groups, each group comprising 6 animals. Throughout the 28–d treatment period, the first group was administered distilled water (2% DMSO), and the second and third groups were administered 100 mg·kg^{-1} and 200 mg·kg^{-1} of the HMEHR, respectively, dissolved in distilled water with 2% DMSO. All administrations were done once daily via oral gavage. The body weight of the experimental animals was recorded on d 1, 7, 14, 21, and 28 of treatment.

Behavioral tests

Kondziela's inverted screen

One d before the euthanasia, some different aspects of behavior, such as muscular strength, were evaluated using adapted tests. These tests help to see if there is a toxic effect of the extract on the nervous system.

To make sure the animals were appropriately awake, they were brought into the experimental room 15 min before testing. This test was published in 1964 and used to assess coordination and neuromuscular diseases [17]. The test was carried out using an inverted screen, which is a 43 cm square of wire mesh, encircled by a 4 cm deep wooden beading. The square is made up of 12 mm squares of 1 mm diameter wire. The time the rat fell is noted and used to calculate its score [18].

Biochemical and hematological parameters

At the end of the experiment, blood was taken via retro–orbital puncture using a capillary to detect possible toxic effects of the extract.

For the biochemical analysis, Heparin tubes were used to collect the blood. and centrifuged (Sigma, 6–16 S, Germany) at 3000 g for 10 min, and plasma was taken to evaluate different biochemical parameters (glucose, creatinine, urea, Alanine Aminotransferase (ALAT), Aspartate Aminotransferase (ASAT), total cholesterol, triglycerides, total protein, albumin, calcium, and phosphate) using a serum biochemistry analyzer (Roche, COBAS C 502, Switzerland).

Blood was also collected in EDTA tubes (Ethylenediaminetetraacetic acid tubes) to evaluate different hematological parameters (Neutrophils, Eosinophils, Basophiles, Lymphocytes, Monocytes, White Blood Cells (WBC), Red Blood Cells (RBC), Hematocrit (HCT), Hemoglobin (HGB), Platelets (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) using the Cellular Analysis Line (Mindray, CAL 8000, China).

Relative organ weight

After euthanasia, the kidneys and liver were dissected out carefully and weighed in g. Afterwards, each animal's relative organ weight was determined using the formula:

$$Relative\ organ\ weight\ = \frac{Absolute\ organ\ weight\ (g)}{Body\ weight\ of\ rat\ on\ sacrifice\ day\ (g)} \times 100$$

Histopathological study

Samples of liver and kidney were collected for histopathological studies. They were washed in normal saline and fixed immediately in 10% formalin. They were then inspected under a microscope (Optika Srl, DM–25, Italy) after being fixed in paraffin, cut into thick sections of 5μm, and stained with hematoxylin and eosin [19] .

Statistical analysis

The data are expressed as mean ± standard deviation. The results were analyzed using a *t*–test of Student and by one–way analysis of variance (ANOVA) followed by Tukey post–hoc test; the differences between groups were considered to be statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

The extract yield was calculated to be 15.52%. The total content of polyphenols was estimated using Gallic acid calibration line, and it was 132.53 ± 2 μg GAE·mg^{-1} of extract, and the total content of flavonoids was estimated using Quercetin calibration line, and it was 96.11 ± 3.65 μg QE·mg^{-1} of extract (TABLE I). The results were significantly higher than those found by Sicari *et al.* [20] in Italy; this may be due to the extraction method and the environmental effects. Vitiello *et al.* [11] found that *H. radiata* is a good source of sugars, polyphenols, polyunsaturated fatty acids, and amino–acids by using LC–HR–Orbitrap/ESI–MS and RMN.

A single method cannot fully evaluate the antioxidant capacity because various antioxidant compounds can function via various mechanisms. Because of this, the investigation of complicated antioxidant activities frequently uses a variety of techniques [21]. For this, DPPH scavenging and the reducing ability (FRAP) tests were used for the evaluation of *H. radiata* antioxidant activity. The extract shows a good antioxidant potential in both tests, and the results were presented in TABLE I. The HMEHR and ascorbic acid's

TABLE I
Total content of polyphenols, flavonoids, and antioxidant activity of HMEHR using two methods DPPH (presented in IC_{50}) and Reducing ability (presented in A0.5)

	Total polyphenols	Total flavonoids	DPPH assay (IC_{50})	Reducing ability (A0.5)
HMEHR	132.53 ± 2	96.11 ± 3.65	50.74 ± 2.01	76.60 ± 1.22
Ascorbic acid	–	–	5.42 ± 0.16	5.61 ± 0.33

HMEHR: Hydro–methanolic extract of *Hyoseris radiata* L., DPPH: 2,2–diphenyl–1–picrylhydrazyl, IC_{50}: Inhibitory Concentration 50, A0.5: Absorbance 0.5

capacity to scavenge DPPH radicals resulted in a notable decrease in their amount. The extract shows an important effect with IC_{50} = 50.74 ± 2.01 µg·mL^{-1}, but it remains less than that of the standard IC_{50} = 5.42 ± 0.16 µg·mL^{-1}. For the test of reducing ability, results show that our extract can reduce Fe^{3+} to Fe^{2+}. The extract has an A0.5 = 76.60 ± 1.22 µg·mL^{-1} but this value remains lower than that of ascorbic acid, which has an A0.5 = 5.61 ± 0.33 µg·mL^{-1}.

According to Vitiello *et al.* [11] the antioxidant effect of *H. radiata* may be due to the presence of many phytocomplex components such as phenolic compounds, chicoric acid and luteolin derivatives.

Acute toxicity

In acute toxicity experiments, which are frequently employed to offer preliminary information on the nature of toxic material, and In order to calculate the maximum non–lethal or minimum lethal dose, and accurately describe how harmful therapeutic herbs are [22]. Animals were observed, and the one oral dosage of the HMEHR at a dose of 2 g·kg^{-1} showed no symptoms of toxicity or mortality in Wistar rats during the experiment period (14 d). Every animal's body weight was recorded once a week, and the curve of body weight revolution (FIG.1) shows that there is no significant difference between the groups. With an LD_{50} greater than 2 g·kg^{-1}, the extract is classified as belonging to category 5 of the worldwide Harmonization System of Chemical Substances, according to the OECD guidelines.

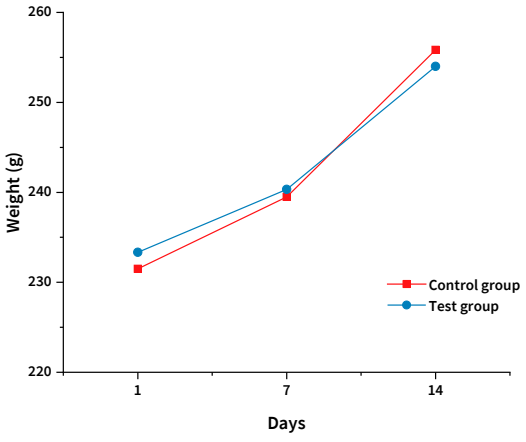

FIGURE 1. Body weight of rats treated orally with HMEHR. Values expressed as mean. n = 6 animals / group

Subacute toxicity

The possibility of being subjected to a particular hazard at different levels is frequently taken into account when measuring risk in plant toxicity research [23].The repeated oral administration of the HMEHR during 28 d with 2 doses 100 mg·kg^{-1} and 200 mg·kg^{-1} did not cause any signs of toxicity or mortality. The body weight of all animals was monitored at the start of the experience and once every week and the results were presented in FIG. 2. Body weight changes were employed as a marker of the possible negative effects of the extract that was given; the mean BW increased normally and progressively for both the treatment and control groups.

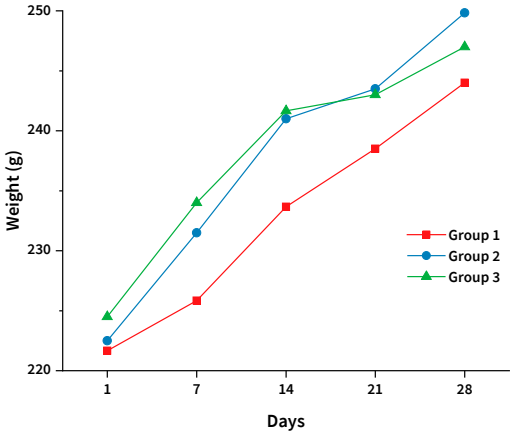

FIGURE 2. Body weight of rats treated by oral gavage with Hydro–methanolic extract of *Hyoseris radiata* L. for 28 days (subacute toxicity). Values expressed as mean. n = 6 animals/ group. Group 1: control group, group 2: treated with 100 mg·kg^{-1} of HMEHR, Group 3: treated with 200 mg·kg^{-1} of HMEHR

Kondziela's inverted screen

It is one of the most used behavioral tests. This test was used to asses coordination and neuromuscular diseases that may be caused by different drugs [17]. The test's findings indicate that the three groups do not significantly differ from one another (FIG. 3). These findings support the neuromuscular safety of the extract at the tested doses, and indicates that repeated administration of the extract did not adversely affect neuromuscular strength in rats.

Biochemical and hematological parameters

After taking blood samples, different hematological parameters were tested. The bloodstream is the main way that foreign materials and various nutrients are moved throughout the body. Therefore, some blood compounds such as RBC, WBC, PLT, and hemoglobin are frequently exposed to different toxins [24]. No significant differences in various hematological parameters were noted between the control group and the two treated groups (TABLE II).

For the results of biochemical parameters, mean values for all parameters are shown in TABLE III. Enzymes, such as ALAT, ASAT, are commonly used as an indicator of liver toxicity. Additionally,

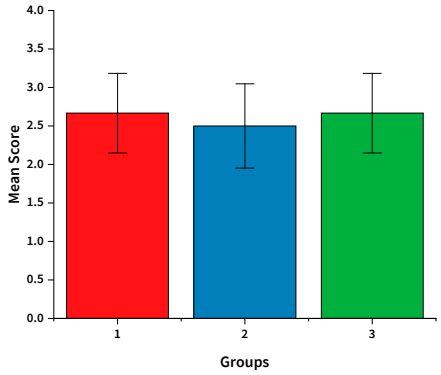

FIGURE 3. Kondziela's inverted screen. Group 1: score of control, Group 2: score of the group treated with 100 mg·kg^{-1} of HMEHR, Group 3: score of the group treated with 200 mg·kg^{-1} of HMEHR

TABLE II
Hematological parameters measured during the subacute toxicity. Group 1: control group, Group 2: treated with 100 mg·kg^{-1} of HMEHR, Group 3: treated with 200 mg·kg^{-1} of HMEHR

Hematological parameters	Group 1 (Control)	Group 2 (100 mg·kg^{-1})	Group 3 (200 mg·kg^{-1})
WBC	4.29 ± 0.95	4.73 ± 0.67	5.46 ± 1.58
Neutrophils	1.02 ± 0.3	1.31 ± 0.31	1.24 ± 0.24
Eosinophils	0.11 ± 0.03	0.15 ± 0.15	0.08 ± 0.03
Basophils	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Lymphocytes	2.98 ± 0.85	3.11 ± 0.66	3.97 ± 1.44
Monocytes	0.18 ± 0.03	0.15 ± 0.06	0.16 ± 0.09
RBC	9.06 ± 0.31	9.19 ± 0.61	9.05 ± 0.33
HGB	17.13 ± 0.56	16.93 ± 0.93	16.90 ± 0.26
HCT	51.18 ± 1.61	50.18 ± 3.44	50.03 ± 3.09
MCV	56.53 ± 1.66	54.58 ± 2.03	55.25 ± 2.03
MCHC	33.48 ± 0.14	33.83 ± 0.45	33.87 ± 0.63
PLT	879.83 ± 111.31	912.67 ± 237.06	767.50 ± 73.46

Values were expressed as mean ± SD (n = 6). HMEHR: Hydro–methanolic extract of *Hyoseris radiata* L.

TABLE III
Biochemical parameters were measured during the subacute toxicity. Group 1: control group, group 2: treated with 100 mg·kg^{-1} of HMEHR, Group 3: treated with 200 mg·kg^{-1} of HMEHR

Parameters	Group 1 (Control)	Group 2 (100 mg·kg^{-1})	Group 3 (200 mg·kg^{-1})
Urea	0.25 ± 0.06	0.24 ± 0.04	0.27 ± 0.04
CREA	4.83 ± 0.57	4.85 ± 0.59	4.52 ± 0.17
ASAT	117.67 ± 26.6	94.33 ± 16.89	98.67 ± 9.99
ALAT	52.17 ± 15.30	48.83 ± 9.49	41.00 ± 9.14
Glucose	1.02 ± 0.1	0.94 ± 0.16	0.98 ± 0.11
Total Cholesterol	0.76 ± 0.09	0.83 ± 0.15	0.73 ± 0.06
Triglycerides	0.86 ± 0.25	0.61 ± 0.08*	0.61 ± 0.09*
Total protein	62.58 ± 1.58	65.00 ± 2.30	61.47 ± 3.06
Albumin	48.67 ± 1.18	49.68 ± 1.11	46.95 ± 2.53
Calcium	111.67 ± 3.01	115.00 ± 2.53	114.00 ± 3.24
Phosphate	65.67 ± 8.11	75.17 ± 11.55	78.83 ± 7.08

Values were expressed as mean ± DE (n = 6). *: *P*<0.05. HMEHR: Hydro–methanolic extract of *Hyoseris radiata* L.

Creatinine and Urea levels are sensitive markers of renal function [25]. The evaluation of biochemical parameters shows that there are no significant differences between control (Group 1) and treated groups in ALAT, ASAT, Urea, Creatinine, Glucose, Total cholesterol, Total protein, Calcium, and Phosphate parameters. A significant decrease in triglyceride was recorded in treated groups with 100 and 200 mg·kg^{-1} BW of extract. Several bioactive compounds, including polyphenols, flavonoids, saponins, and terpenoids, are known to enhance lipid metabolism or reduce hepatic lipid synthesis by inhibiting lipogenesis or promoting fatty acid oxidation. The hydro–methanolic extract of *H. radiata* may act through one or more of these mechanisms. Moreover, a study conducted by Sicari *et al.* [20] reported that *H. radiata* leaves exhibit strong pancreatic lipase inhibitory activity, even greater than that of other plant species. This suggests that the observed reduction in triglyceride levels could be attributed, at least in part, to the inhibition of pancreatic lipase, leading to reduced dietary fat absorption and contributing to the regulation of lipid metabolism in vivo.

Relative organ weight

For each animal, the organ weight of the kidney and liver was calculated relative to their body weight and the results were presented in (FIG. 4). Some research indicate that the measure of organ weight can be useful to check the health and well–being of animals [26], and that herbal products can be harmful to specific organs that are vital to the animal's body, such as the liver and kidneys [25]. The study's findings showed no significant differences in the kidney and liver's relative organ weights.

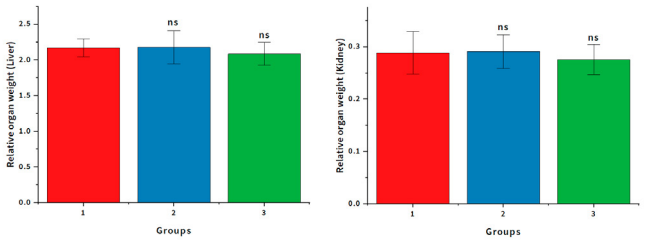

FIGURE 4. Relative organ weights of the liver and kidney. Group 1: control, Group 2: treated with 100 mg·kg^{-1} of HMEHR , Group 3: treated with 200 mg·kg^{-1} of HMEHR. Values were expressed as mean ± SD (n = 6). ns: no significant

Histopathological study

The histological examination of the liver and kidney, which are responsible for the elimination of xenobiotics and detoxification of the organism revealed no signs of toxicity or dysfunction in the kidney. A mild congestion was observed in the liver of rats treated with 200 mg·kg^{-1} BW of the extract (FIG. 5). However, no associated histopathological alterations, such as necrosis or inflammation, were noted, suggesting that the extract did not induce significant hepatic toxicity.

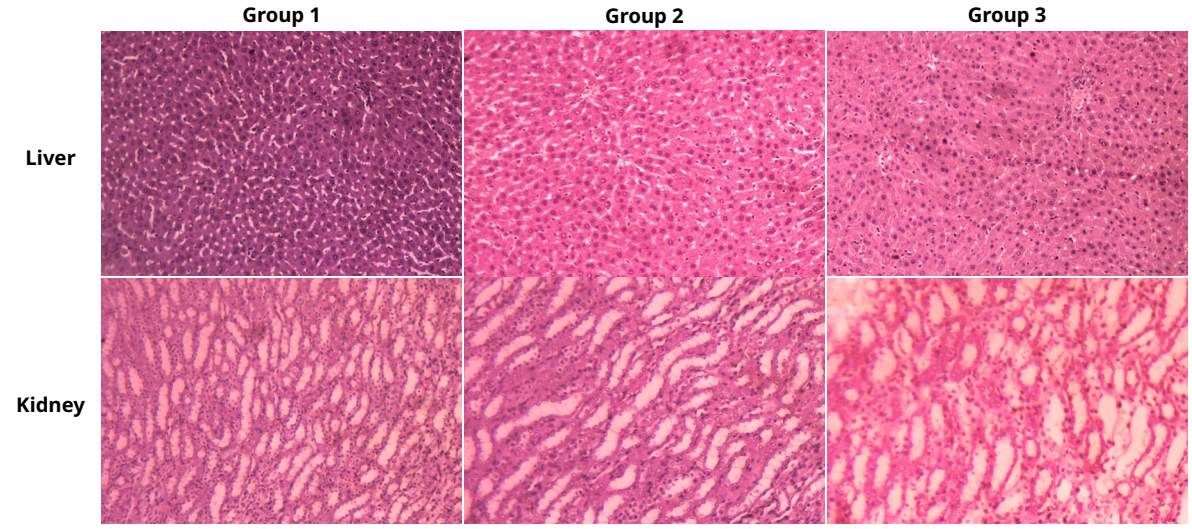

FIGURE 5.Histopathological analysis of rats' kidney and liver in subacute toxicity (10×). Group 1: control, Group 2: treated with 100 mg·kg^{-1}, Group 3: treated with 200 mg·kg^{-1} of HMEHR

CONCLUSION

The results of this study indicate that the hydro–methanolic extract of *Hyoseris radiata* L. is rich in polyphenols such as flavonoids. The extract shows an important antioxidant activity in both tests, DPPH and reducing ability. Based on LD_{50} values for oral routes, the extract's results show no acute toxicity. Although, the 28 d subacute toxicity test revealed no serious toxic effects on important organs, on hematological parameters, and biochemical parameters. A significant decrease in triglyceride was recorded for both evaluated concentrations. To validate these results, more studies with higher doses, other delivery methods, and longer periods are required. Comprehensive evaluations of the toxicity of bioactive components, neurotoxicity, and reprotoxicity should also be carried out. The present finding explains the continuous use of *Hyoseris radiata* L. different extracts in herbal medicine.

Conflicts of interest

The authors declare no conflict of interest.

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