INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.12.1.2022.12(1)

EVALUATION OF ACUTE TOXICITY OF *HELICHRYSUM ARENARIUM* AQUEOUS EXTRACT IN SWISS ALBINO MICE

Mounira Merghem[1], Meriem Hani[2*] and Saliha Dahamna[1]

[1]Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature and Life Sciences, Ferhat Abbas University, Sétif 1, Algeria.

[2]Laboratory of Natural Resource Valorisation, Faculty of Nature Life Sciences, Ferhat Abbas University Setif-1,19000, Algeria.

ABSTRACT

Helichrysum arenarium is a medicinal plant from the *Astéraceae* family, used as an antimicrobial and for the treatment of digestive diseases such as indigestion and anorexia. The aim of this study is to evaluate the acute toxicity of *Helichrysum arenarium*aqueous extract. The mice were divided into three groups each with 3 mice, the first group received distilled water, the second and third groups were administered orally at single dose of 2000 mg/kg and 5000 mg/kg respectively, and then observed individually for the first four hours, then over a period of 24 hours and at least once daily for 14 days. The results showed that a single administration of the aqueous extract of *Helichrysum arenarium* up to a dose of 5000 mg/kg did not cause any mortality or signs of toxicity, an increase in food consumption and body weight of treated mice compared to control, No change in organs weights. The biochemical study also showed a slight increase in ALT in rats treated with 5g/kg, a slight decrease in the levels of AST in rats treated with 2g/kg and a slight decrease in the levels of Créa in rats treated with 2g/kg and 5g/kg in comparison to the control rats.

Keywords: *Helichrysum arenarium,* Acute Toxicity, Aqueous extract and biochemical parameters.

INTRODUCTION

Medicinal plants are used world wide to treat many diseases, and new drugs continue to be developed through research from these plants[1]. There is also an emerging increase in the consumption of herbal formulations by the public because of the strong belief that these products are natural; hence, they are safe for the treatment of ailments[2]. Nevertheless, the latest surveys have indicated that many of these products used in traditional medicine showed adverse effects. Since safety continues to be a major issue with the use of medicinal plants, it is important to conduct toxicity studies on them to ascertain their safety profile. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used in animals or humans is an important aspect of its assessment for potential toxic effects[3].

Toxicology is the important part of pharmacology which deals with the undesirable effect of phytocompounds on living organisms previous to the use as drug or chemical in clinical use. Several studies are concentrated on toxicity analysis so as to determine the safeness of medicinal plants and their products. Toxicity analysis is essential, as some herbs consumed might have some toxic effects and many reports have been published for toxicity caused due to long term consumption of herbs[4].

The genus Helichrysum, belonging to the family Asteraceae is represented by approximately 300 species in the world[5]. Some members of this genus are used in traditional medicine and known for their important activities[6]. *Helichrysum arenarium* is one of the most widely consumed plants among different species of the genus *Helichrysum. Helichrysum arenarium* has long been used in traditional medicine as an herbal tea for the treatment of various health-related problems such as fever and nervousness, as well as gall-bladder and urinary-tract diseases[7]. Chemical compounds present in the inflorescences protect the liver from toxins; they are also effective antioxidants, antiseptics[8]. antimicrobial, antiradical[9].

MATERIALS AND METHODS

Plant Material

Helichrysum arenarium plant material was collected from Bougaa région, Wilaya of Sétif Northeast of Algeria.

Animal Material

Male*Albino Wistar* mice weighing between30 and 45 gwere used for acutetoxicity. The animals were obtained from Pasteur Institute(Algiers, Algeria). These animals were kept in theanimal house of the faculty of Nature and Life Sciences,University of Sétif, at a temperature of 20°C and a photoperiod cycle of 12 hours light/dark. The animals werehoused in plastic cages (3 mice per cage) and hadfree access to standard commercial diet and tap water.

Preparation of aqueous extract

The areal parts were washed in running water, dried and powdered. 50 g of powder was boiled in 500 ml of water for 15 minutes, the resulting was filtered using wattman filter paper and then evaporated in rotary vacuum evaporator.

Acute toxicity study

The acute oral toxicity of extract was evaluated using the procedures described by Organization for Economic Co-operation and Development 425 guidelines, the animals were divided into three groups with 3 animals (3 males). The control group was given distilled water. The second and third groups were given a single dose of 2000 mg/kg and 5000 mg/kg of aqueous extract respectively. The animals werefasted (4h) with free access to water priorto administration of single doses of the extract dissolved in distilled water. The general behavior of the mice was continuously monitored after dosing, periodically during the first 24 h (with special attention given during the first 4 hours), and then daily thereafter, for a total of 14 days.

At the end of the treatment, animals were fasted overnight, but allowed access to water and libitum. They were subsequently anesthetized with diethyl ether and blood samples were obtained by retro-orbital puncture and collected in tube containing heparin and centrifuged at 4000 r/min at 4°C for 15 minutes to obtain plasma (stored at −20°C until analysis).

Body Weight, Food and Water Consumption

The body weight of each mice was recorded once weekly and the amount of food and water consumed was measured from the quantity supplied and theamount remaining after 24 hours for 2 weeks of the study period.

Biochemical analysis

Biochemical analysis was performed using an automatic analyzer (Beckman). Parameters included: Créatinine (Créa); Urée ;aspartate aminotransferase (AST); alanine aminotransferase(ALT).

Organ weights

After the sacrifice of all animals, the kidneys, liver, heart,lungsand spleenwere carefully removedand weighed individually.

Statistical analysis

The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to assess differences between groups.

RESULTS AND DISCUSSION

Mortality and signs of toxicity

No mortality and signs of toxicity were observed in any of the treated rats at the dose level of 2000 mg/kg and 5000 mg/kg body weight of *Helichrysum arenarium*aqueous extract (Table 1).Therefore, we can estimate that the LD_{50} value was higher than 5000 mg/kg.

Body weight changes

Bodyweight and general behavior of animals were frequently assessed to indicate the occurrence of a toxic effect or a lack of a toxic effect[10]. The results showed that there was a slight increase in body weight of the treated groups compared to the control group (Table 2).

Food and water consumption

Determination of food intake and water consumption is important in the study of safety of a product with therapeutic purpose,as proper intake of nutrients is essential to the physiological status of the animal and to the accomplishment of the proper response to the drugs tested[11].

The amount of food and water consumed was measured daily from the quantity of food and water supplied and the amount remaining after 24 h. The results showed an increase in food consumption in group treated with 2g/kg and a slight increase in group treated with 5g/kg of *Helichrysum arenarium* aqueous extract compared to the control group (Table 3). However, no difference in water consumption between groups.

Biochemical analysis

The values for the biochemical parameters in treated and control animals are presented in Table 4. This values showed a slight increase in the levels of serum ALT in rats treated with 5g/kg, a slight decrease in the levels of AST in rats treated with 2g/kg and a slight decrease in the levels of Créa in rats treated with 2g/kg and 5g/kgin comparison to the control rats. Nevertheless, the level of these marker enzymes for both the treated and control group falls within the normal range.

The biochemical parameters levels indicates physiological condition. The increase and decrease of biochemical parameters can convey indications regarding toxicity of specific organs[12]. The liver and kidney are vital organs that play significant roles in metabolic activities in the body[13]. Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST), are known as sensitive biomarkers of hepatocellular function[14]. In fact, ALAT and ASAT are serum enzymes markers synthesized in the liver. ALT is localised in the cell cytoplasm while AST is found both in mitochondria and cytoplasm [15].Changes can occur in the levels of these enzymes when there are changes in hepatic cellular permeability, damage to the hepatocytes, or necrosis[16].

On the other hand, renal function can be assessed by changes in urea and creatinine, and an increase in these parameters indicates possible damage to renal function[17].

Organs weight

Organ weight also is an important index of physiological andpathological status in animals. The relative organ weight is fundamental to diagnose whether the organ was exposed to the injury or not. The heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicant[18].

organs weight are shown in Table 5. There were no changes in the organs weight of the treated animals compared to the control group.

Table 1: Mortality and signs of toxicity of the *Helichrysum arenarium* administered orally as one single dose in mice

Dose (g/kg)	Death (D/T)	Adverse Effects
0	0/3	Normal
2	0/3	Normal
5	0/3	Normal

Table 2 : Effect of *Helichrysum arenarium* aqueous extract on body weight in mice

Day	control	2000 mg/kg	5000 mg/kg
1st Day Body Weight (g)	33,79± 3,53	37,26± 3,13	34,37±2,97
7th Day Body Weight (g)	33,75± 4,19	40,66± 3,23	37,93± 7,81
14th DayBody Weight (g)	33,89± 5,03	42,16± 14,95	42,51±1,77

Values are presented as mean ± SD; N= 3.

Table 3 : Effect of *Helichrysum arenarium* aqueous extract on food and water consumption in mice

Food and Water consumption			
	Control	2000 mg/kg	5000 mg/kg
Food (g)	36,80 ±1145	40 ±20	5141± 18,90
Water (ml)	47,20± 11, 0341,	67±19,40	50±20,18

Values are presented as mean ± SD; N= 3.

Table 4 : Effect of *Helichrysum arenarium* aqueous extract on biochemical parameters of mice

Parameters	Control	2000 mg/kg	5000 mg/kg
ALT(UI/L)	29±16,86	38,5±31,62	58± 32,04
AST(UI/L)	119,66±96, 38	85±49,07	138± 84,45
Créa (mg/L)	5,4±3,09	2,77±1,62	2,64±0,61
Urée (mg/L)	0,45±0,09	0,67±0,42	0,7± 0,26

Values are presented as mean ± SD; N= 3.

Table 5 : Effect of *Helichrysum arenarium* aqueous extract on organ weight in mice

Organ (g)	Control	2000 mg/kg	5000 mg/kg
Liver	2,31± 0,47	1,56± 0,91	1,78± 0,15
Kidneys	0,27± 0,04	0,29± 0,10	0,31± 0,05
Lungs	0,28± 0,09	0,33± 0,14	0,30± 0,07
Heart	0,18± 0,03	0,17± 0,36	0,17± 0,01
Spleen	0,12 ± 0,01	0,12± 0,08	0,12± 0,03

Values are presented as mean ± SD; N= 3.

CONCLUSION

Acute toxicity study of the aqueous extract prepared from the aerial part of *Helichrysum arenarium* was assased by oral administration at the doses of 2 g/kg and 5 g/kg in male albino wistar mice. The results showed that this plant did not cause any deaths or other signs of toxicity, this indicates that this plant is not toxic.

REFERENCES

1. Al-AfifiN A, AlabsiA M, Bakri MM and Ramanathan A. Acute and sub-acute oral toxicity of Dracaena cinnabari resin methanol extract in rats. BMC Complementary and Alternative Medicine. 2018;18:50.
2. Olaniyan JM, Muhammad HL, Makun HA, Busari MB and Abdullah AS. Acute and sub-acute toxicity studies of aqueous and methanol extracts of Nelsonia campestris in rats. Journal of Acute Disease. 2016;5(1):62–70.
3. Ugwah-OguejioforC J, OkoliC O, UgwahM O, UmaruM L, OgbulieC S, MsheliaH E, UmarM and NjanA A. Acute and sub-acute toxicity of aqueous extract of aerial parts of Caralluma dalzielii N. E. Brown in mice and rats. Heliyon. 2019;5:1-20.
4. Subha D and Geetha N. Evaluation of acute toxicity of the methanolic extract of Tanacetum parthenium L. in albino wistar rats. Journal of Scientific and Innovative Research. 2017;6(3): 113-115.
5. Tepe B, Sokmen M, Akpulat HA and Sokmen A. In vitro antioxidant activities of the methanol extracts of four Helichrysum species from Turkey. Food Chemistry. 2005;90:685–689.
6. Kupeli E, Tosun A and Bahadir O. Evaluation of anti-inflammatoryand antinociceptiveactivities of Helichrysum gaertner species (Asteraceae). Turk J Pharm Sci. 2006 ;3(3):141-149.
7. Ivanovic M, Albreht A, Krajnc P, Vovk I and Razborsek MI. Sustainable ultrasound-assisted extraction of valuable phenolics from inflorescences of Helichrysum arenarium L. using natural deep eutectic solvents. Industrial Crops and Products. 2021;160:113102.
8. Sawilska AK and Jendrzejczak E. Efficiency of Sandy Everlasting [Helichrysum arenarium (L.) Moench] cultivation from in vitro seedlings and achenes. Industrial Crops and Products. 2013;43:50–55.
9. Liu X, Jing X and Li G. A process to acquire essential oil by distillation concatenated liquid-liquid extraction and flavonoids by solid-liquid extraction simultaneously from Helichrysum arenarium (L.) Moench inflorescences under ionic liquid microwave mediated. Separation and Purification Technology. 2019;209:164–174.
10. Sireeratawong S, Lertprasertsuke N, Srisawat U, Thuppia A, Ngamjariyawat A, Suwanlikhid N and Jaijoy K. Acute and subchronic toxicity study of the water extract from Tiliacora triandra (Colebr.) Diels inrats Songklanakarin J Sci Technol. 2008;30:611–619.
11. Subramanion LJ, Zuraini Z, Yeng C, Yee Ling L, Lachimanan YL and Sreenivasan S. Acute oral Toxicity of Methanolic Seed Extract of Cassia fistulain Mice. Molecules. 2011;16:5268-5282.
12. Almamy K, Geoffroy GO, Sylvain I, Noufou O, Adama K, Amadou T, Innocent PG and Hamidou HT. Acute and sub-acute oral toxicity studies of aqueous extract of Securidacalonge pedunculatafresen (Polygalaceae) root barks in rodents. Int J Adv Res. 2016;4(12):550-558.
13. Mukinda JT and Syce JA. Acute and chronic toxicity of aqueous extract of Artemisia afra in rodents. J Ethnopharmacol. 2007;112(1):138-44.

14. Traesel GK, de Souza JC, de Barros AL, Souza MA, Schmitz WO, Muzzi RM,Oesterreich S A and Arena AC. Acute and subacute (28 days) oral toxicity assessment of the oil extracted from Acrocomia aculeata pulp in rats. Food Chem Toxicol. 2014;74:320–325.
15. Prosper BN, Jules KR, Modeste W and Ntiokam D. Acute and subacute toxicity studies of Zinginber officinalis roscoe essential oil on mice (swiss) and rats (wistar). African Journal of Pharmaceutical Sciences and Pharmacy. 2010;1(1).
16. Sujith K, Darwin R and Suba V. Toxicological evaluation of ethanol extract of Anacyclus pyrethrum in albino Wistar rats. Asian Pac J Trop Dis. 2012;2:437-441.
17. Ezeja MI, Anaga AO and Asuzu IU. Acute and sub-chronic toxicity profile of methanol leaf extract of Gouania longipetala inrats. J. Ethnopharmacol. 2014;151(3):1155–1164.
18. Dybing E, Doe J, Groten J, Kleiner J and O'Brien J. Hazard characterization of chemicals in food and diet. dose response, mechanism and extrapolation issues. Food Chem Toxicol. 2002;42:237-282.