Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Acute Toxicity Study of *Centaurium Erythraea* Aqueous Extract in Swiss Albino Mice

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ARTICLE DETAILS

Article history:
Received on 27 May 2020
Modified on 21 July 2020
Accepted on 29 July 2020

Keywords:
Centaurium Erythraea,
Acutetoxicity,
Biochemical Parameters,
Histopathology Examination.

ABSTRACT

The plant *Centaurium erythraea* is a medicinal plant used for the treatment of the many diseases. The aim of present study is to investigate the acute toxicity of aqueous extract of Centaurium erythraea in *Swiss albino* mice. mice were administered a single dose of 2000 mg/kg and 5000 mg/kg orally and then observed individually for the first four hours, then over a period of 24 hours and at least once daily for 14 days. Results showed that a single administration of the aqueous extract of *Centaurium erythraea* up to a dose of 5000 mg/kg did not cause any mortality or signs of toxicity in the mice tested during the observation period. There were no significant differences in body weight, food consumption, absolute organ weights between controls and treated animals. Biochemical analysis showed no differences in parameters examined. No histological changes were observed in organs of treated mice compared to control. These results indicates the safety usage of extract from this plant in traditional medicine.

INTRODUCTION

plant-based products play a key role in the primary health care of about 80-85% of the world's population. Medicinal plants has increasing gained public and professionals acceptance due to progresses in the understanding of the mechanisms by which these plants positively influence health and quality of life [1]. Although medicinal plants have several therapeutic virtues, they are not free from any danger of intoxication. Several researchers have pointed out the potential toxicity, as well as the risks associated with the use of certain species of plants [2]. Therefore, it is important to evaluate the adverse effects of these plants and their preparations to increase the confidence in its safety to human, particularly for use in the development of pharmaceutical products [3].

Centaurium erythraea is a plant belonging to the Gentianaceae family, known as common centaury [4]. This is a biennial plant with basal leaf rosette, growing to the height of 15–24 cm.

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Its stems are branched, bearing clusters of pink or red flowers. Centaury grows in dry and sandy places in Europe, Western Asia, North Africa and North America [5].

Centaury is a traditional medicinal species that has been used for digestive, stomachic, tonic, depurative, sedative and antipyretic purposes [6], Renal, hepatic, respiratory, and rheumatic diseases. It is also taken as an antidiabetic agent and to treat cardiovascular diseases, namely hypertension [7]. The antiinflamatory and antipuretic effects of an aqueous extract of the plant have already been observed experimentally in rats [8]. The dried aerial parts of Centaury have been informed to be used in the treatment of anorexia and dyspepsia [6].

Previous phytochemical studies on *Centaurium erythraea* revealed the presence of a variety of plant secondary metabolites, including centauroside, centapiricin, flavonoids, gentiopicrin, gentiopicroside, isocumarin, phenolic acids and their derivatives, swertiamarin, terpenoids and xanthones [9].

The present study aims to determine the acute toxicity of aqueous extract of *Centaurium erythraea*. The acute oral toxicity testing was carried out on both sexes of mice under the

Organization for Economic Cooperation and Development (OECD) guidelines.

MATERIALS AND METHODS

Plant Material

Centaurium erythraea L. plant material was collected from Babor région, Wilaya of Sétif Northeast of Algeria.

Animal Material

Albino Wistar mice of both sexes weighing between 25 and 40 g were used for acute toxicity. The animals were obtained from Pasteur Institute (Algiers, Algeria). These animals were kept in the animal 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 were housed in plastic cages (3 mice per cage) and had free 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 6 animals (3 males and 3 females). The control group was given normal saline. The second and third groups were given a single dose of 2000 mg/kg and 5000 mg/kg of aqueous extract respectively. The animals were fasted (4h) with free access to water prior to 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 serum (stored at −20°C until analysis). The organs (kidneys, liver, lungs, heart, stomach and spleen) were weighed and fixed in 10% formalin for histopathological examination.

Body Weight and Food Consumption

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

Blood Analysis

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

Organ Weights

After the sacrifice of all animals, the kidneys, liver, heart, lungs, spleen and stomach were carefully removed and weighed individually (absolute organ weight).

Histopathological Examination

Liver and kidneys were carefully dissected out, and rinsed in 0.9% NaCl, then fixed in the formol (10%), sectioned 5 µm thickness, and embedded in paraffin and stained with hematoxylin and eosin, and examined with a light microscope.

Statistical Analysis

The results are expressed as the mean value ± standard deviation. One-way analysis of variance followed by the Tukey test was performed to assess differences between groups. Differences were considered significant atp < 0.05. Statistical analyses were performed with the aid of the software GraphPad Prism 7®.

RESULTS

Mortality and Signs of Toxicity

In the toxicity study, oral administration of *Centaurium erythraea* aqueous extract at 2000 mg/kg and 5000 mg/kg did not cause any deaths and clinical signs of toxicity in mice. Therefore, we cannot determine LD_{50} from the study. We can estimate that the LD_{50} value was higher than 5000 mg/kg.

Body Weight Changes

In this study there were no significant changes in body weight. The results were mention in Table 1.

Table 1 : Effect of *Centaurium erythraea* aqueous extract on body weight in mice.

Day	Sex	control	2000 mg/kg	5000 mg/kg
1st Day Body Weight (g)	M	36,95 ±1,824	30,91 ± 4,871 ns	37,68 ± 3,693 ns
	F	28,77 ± 2,289	28,61 ± 1,469 ns	29,32 ± 3,058 ns
7th Day Body Weight (g)	M	37,01 ± 3,069	33,48 ± 6,707 ns	38,51 ± 4,434 ns
	F	28,25 ± 2,263	28,37 ± 1,836 ns	29,59 ± 3,476 ns
14th DayBody Weight (g)	M	36,97 ± 4,137	33,34 ± 8,975 ns	39,55 ± 4,810 ns
	F	29,46 ± 3,659	27,43 ± 1,623 ns	30,15 ± 3,890 ns

Values are presented as mean ± SD; N= 3. ns :no significant difference. M: Male F:Female

Food Consumption

The amount of food consumed was measured daily from the quantity of food supplied and the amount remaining after 24 h. The results showed no statistically significant difference between any groups (Table 2).

Table 2 : Effect of *Centaurium erythraea* aqueous extract on Food Consumption in mice.

Sex	Food consumption (g)		
	Control	2000 mg/kg	5000 mg/kg
M	30.14 ± 18	7129.69±9.23	32.20±19.62
F	22.90 ± 13	1415.25±3.53	16.49±2.89

Values are presented as mean ± SD; N= 3. M: Male F:Female

Biochemical Analysis

Table 3, shows the changes of biochemical parameters in the serum of mice induced by *Centaurium erythraea* aqueous extract. There are no significant changes for the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and cholesterol (CHOL) after oral administration of *Centaurium erythraea* aqueous extract.

Organs Weight

Absolute organ weights are shown in Table 4. There were no significant changes in the organs weight of the treated animals compared to the control group.

Table 3 : Effect of *Centaurium erythraea* aqueous extract on biochemical parameters of mice.

Parameters	Sex	Control	2000 mg/kg	5000 mg/kg
ALT(UI/L)	M	44.09±0.0	43,94 ±5,40 ns	46.19±9.07 ns
	F	19.12±2.59	24.74±1.39 ns	29.68±2.17 ns
AST(UI/L)	M	125.7±4.45	137.1±5.09 ns	100.7±3.71 ns
	F	86.43±3.70	89.92±8.64 ns	99.52±0.0 ns
CHOL(g/L)	M	0.40±0.01	0.42±0.01ns	0.46±0.22ns
	F	0.30±0.07	0.31±0.18ns	0.28±0.15ns

Values are presented as mean ± SD; N= 3. ns : no significant difference. M: Male F: Female

Table 4 : Effect of *Centaurium erythraea* aqueous extract on relative organ weight in mice.

Organ (g)	Sex	Control	2000 mg/kg	5000 mg/kg
Liver	M	2.15±0.16	2.01±0.48 ns	2.27±0.25 ns
	F	1.42±0.21	1.40±0.03ns	1.34±0.20 ns
Kidneys	M	0.62±0.02	0.59±0.14 ns	0.69±0.09 ns
	F	0.35±0.08	0.31±0.01 ns	0.34±0.05ns
Lungs	M	0.21±0.01	0.24±0.01ns	0.30±0.05 ns
	F	0.23±0.01	0.24±0.01 ns	0.22±0.01 ns
Heart	M	0.17±0.01	0.16±0.04 ns	0.19±0.02 ns
	F	0.15±0.02	0.13±0.01 ns	0.14±0.01ns
Stomach	M	0.32±0.10	0.30±0.06 ns	0.32±0.02 ns
	F	0.33±0.15	0.28±0.01 ns	0.30±0.07ns
Spleen	M	0.30±0.19	0.39±0.17 ns	0.48±0.01 ns
	F	0.13±0.04	0.13±0.01ns	0.13±0.01 ns

Values are presented as mean ± SD; N= 3. ns : no significant difference. M: Male F: Female

Histological Study

Macroscopic examination of the organs of the animals treated with extract showed no changes in color compared to control. Histopathology results were shown in Fig. 1 to 4. There are no significant morphological changes detected in liver and kidney in all mice from all groups of study.

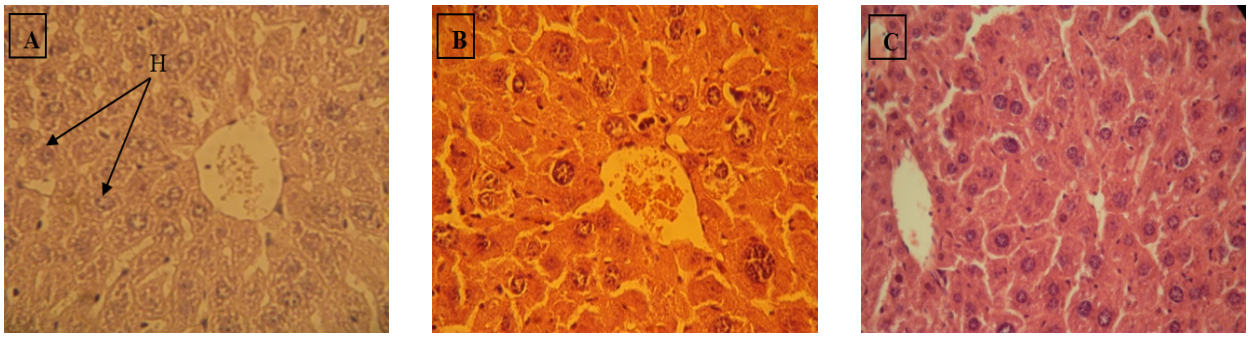

Figure 1 : Effects of *Centaurium erythraea* aqueous extract on liver histomorphology in male mice. (a) Control, (b) treated with 2000 mg/kg, (c) treated with 5000 mg/kg, (H : Hépatocytes).

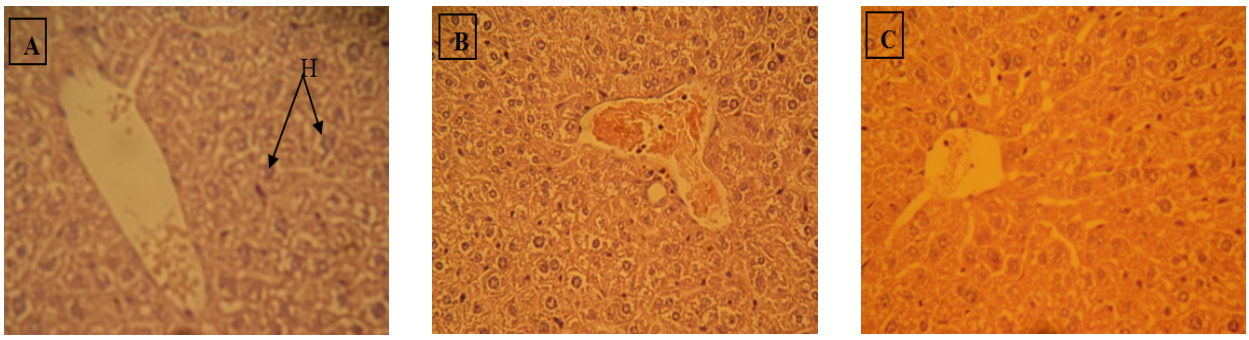

Figure 2 : Effects of *Centaurium erythraea* aqueous extract on liver histomorphology in female mice. (a) Control, (b) treated with 2000 mg/kg, (c) treated with 5000 mg/kg, (H : Hépatocytes).

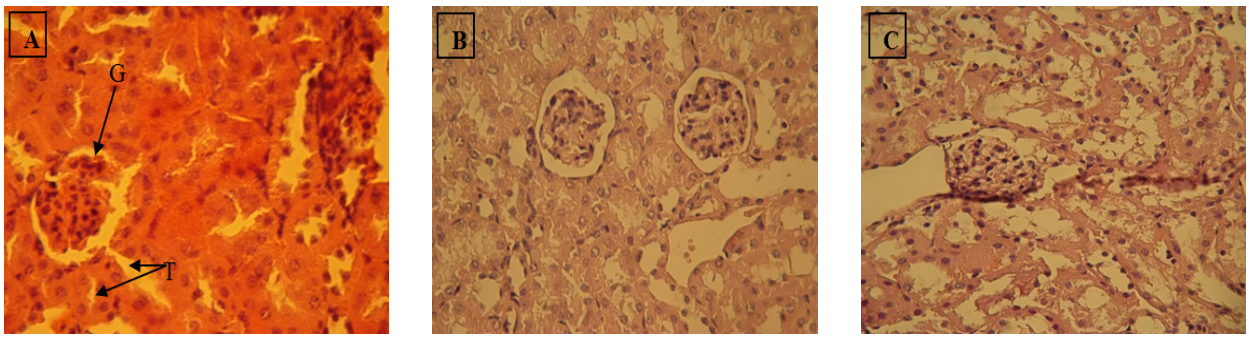

Figure 3: Effects of *Centaurium erythraea* aqueous extract on kidneys histomorphology in male mice. (a) Control, (b) treated with 2000 mg/kg, (c) treated with 5000 mg/kg, (G : Glomérule, T : Tibule).

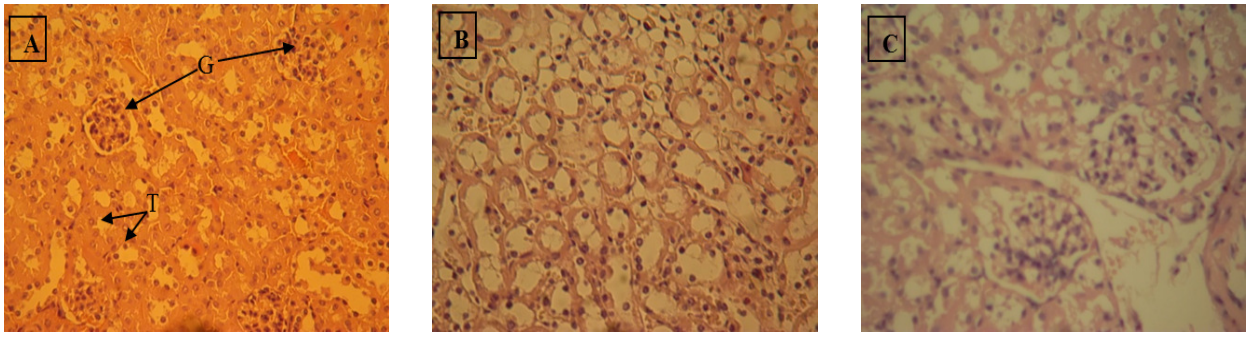

Figure 4: Effects of *Centaurium erythraea* aqueous extract on kidneys histomorphology in female mice. (a) Control, (b) treated with 2000 mg/kg, (c) treated with 5000 mg/kg, (G : Glomérule, T : Tibule).

DISCUSSION

The use of medicinal plants in treatments of various illness is increasing globally [10]. A large number of medicinal plants and botanical drugs are being employed as major therapeutic agents or supplements for treatment of various human diseases [11]. And some have been mistakenly regarded as safe just because they are a natural source [12]. Due to adverse reactions and side effects caused by the administration of these herbal medicines in humans, it is very important to evaluate the safety of medicinal plants and ensure their safe usage [13]. Therefore, further acute oral toxicity study is vitally needed not only to identify the range of doses that could be used subsequently, but also to reveal the possible clinical signs elicited by the substances under investigation [12]. Acute oral toxicity test is one of the pre-clinic test that aims to see toxic effect occured in short period, through single administration each oral or through repeated doses on 24 hours interval [14]. Hence the present study was undertaken to evaluate the acute toxicity of aqueous extract of *Centaurium erythraea*. In the present study no death or signs of toxicity were observed in mice in the 14 days observation period after oral administration of single doses of the aqueous extract of *Centaurium erythraea* at doses of 2000 mg/kg and 5000 mg/kg. This indicate that the LD_{50} of the extract is more than 5000 mg/kg. Accordingto the classification of Hodge and Sterner [15] Chemical substances with a LD_{50} between 5000 and 15000 mg/kg body weight determined after a single oral doses in rats is considered as practically non-toxic in humans. Therefore, the aqueous extract of *Centaurium erythraea* could be considered practically non-toxic. These results are similar to those obtained by Tahraoui et al [16], which showed that no deaths or any signs of toxicity observed after oral administration of single doses of the *Centaurium erythraea* extract at any dose level up to the highest dose tested (15 g/kg BW).

General behavioural changes and body weight are preliminary indicators of early signs of toxicity caused by various chemicals and drugs [17]. A dose, which causes 10% or more reduction in the body weight, is considered to be a toxic dose. It is considered to be the dose, which produces minimum toxic effect, irrespective of whether or not it is accompanied by any other changes [18]. During 14 days of acute toxicity evaluation period, it was observed that food intake was normal with body weight variations. It suggests the normal processing of lipids, carbohydrates and protein metabolism inside animals body because these nutrients play a major role in different physiological functions of the body [19].

Organ weight also is an important index of physiological and pathological 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 [20]. Liver and kidney weights were considered useful in toxicity studies because of its sensitivity to predict toxicity and correlates well with histopathological changes. And thus, it is frequently a target organ of toxicity. In addition, liver is known as primary detoxification organ [21]. And plays an important role in drug elimination but in turn, it can be subject to damage by xenobiotics [22].

The biochemical properties changes could be the indication of alteration or damage in some tissues, organs, and systems of mice [23]. The liver plays an important role in many metabolic processes; any disturbance in the liver would affect the normal level of measurable biochemical parameters in this organ. AST and ALT are marker enzymes present in high concentrations in the liver [24]. Normally, destruction to the liver parenchymal cells will result in an increase of both these enzymes in the blood [20]. There were no changes in the ALT and AST levels, which reveal that the extract did not affect the liver function/ or metabolism. These results are similar to those obtained by Tahraoui et al [16], which showed that repeated oral administration of *Centaurium erythraea* extract (up toa daily dose of 1200 mg/kg BW for 90 days) did not cause significant changes in cholesterol and the liver marker enzymes (ALT and AST).

Histological analysis was done to further confirm the alteration in cell structure of the organs. The histological examination is the golden standard for evaluating treatment related pathological changes in tissues and organs [16]. In the present study, results of acute oral toxicity of *Centaurium erythraea* extract indicated that the extract did not affect the histological structure of organs.

CONCLUSION

The present study was carried out to evaluate the acute toxic effects of aqueous extract prepared from the aerial parts of *Centaurium erythraea* L. This study showed that aqueous

extract did not cause any mortality or other toxicity signs. Acute toxicity study suggested that aqueous extract of the plant is safe up to the dose of 5 g/kg of mice body weight when consumed by oral route.

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