Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60263-9

Acute and Subacute Toxicity study of the Acetone Leaf extract of *Centella asiatica* in Experimental Animal Models

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ARTICLE INFO

Article history:
Received 2 May 2012
Received in revised form 6 May 2012
Accepted 9 August 2012
Available online 28 August 2012

Keywords:
Centella asiatica
acute
subacute toxicity
hematological parameters

ABSTRACT

Objective: To evaluate acute and subacute toxicity of the acetone extract of *Centella asiatica* (Brahmi). **Methods:** Toxicity of *Centella asiatica* was evaluated in Swiss mice after ingestion of the extract during one day (acute model) and during 15 days (subacute model). The Biochemical parameters evaluated included creatinine, calcium, inorganic phosphorous, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed using commercial kits. **Results:** The results of the present investigation revealed that the LD50 of the extract is higher than 4000mg/kg and subacute treatment did not shows any change in corporal weight and hematological parameters. However, a change in liver weight but not in hepatic enzymes was observed. This suggested that the liver function is not altered by *Centella asiatica*. Some changes in the creatinine content were observed but could not be relative with the extract dose. **Conclusions:** The results suggest that the plant seems to be destitute of toxic effects in mice.

1. Introduction

Herbs have been used throughout human history as sources of food, medicines, beauty enhancers, and fragrances. It has been traditionally used as a system of medicine to promote health and well-being, and relieve ailments using a holistic approach (1). *Centella asiatica* is a small herbaceous annual plant of the family Apiaceae, and is native to India, Sri Lanka, northern Australia, Indonesia, Malaysia, and other parts of Asia. It is used as a medicinal herb in Ayurvedic medicine, traditional African medicine, and traditional Chinese medicine. Gotu kola is a mild adaptogen, is mildly antibacterial, anti-viral, anti-inflammatory, anti-ulcerogenic, anxiolytic, a cerebral tonic, a circulatory stimulant, a diuretic, nervine and vulnerary (2). It also possesses antioxidant, cognitive enhancing and antiepileptic properties. Acute ischemia followed by reperfusion is known to bring about biochemical and histopathological alterations. The alcoholic extract of *Centella asiatica* leaves was found to increase proline incorporation and stimulate collagen biosynthesis (3). The aqueous extract of *Centella asiatica* possesses antioxidant, cognitive-enhancing and antiepileptic properties (4). Ayurvedic medicine has effectively used CA in the treatment of inflammation, anemia, asthma, blood disorders, bronchitis, fever, urinary discharge and splenomegaly. Several scientific reports have documented *Centella asiatica* ability to aid wound healing. Upon treatment with *Centella asiatica*, maturation of the scar is stimulated by the production of type I collagen. The treatment also results in a marked decrease in inflammatory reaction and myofibroblast production (5). Many of the plants used today were known to the people of ancient cultures throughout world & these were valued their preservative & medicinal properties. It has been observed hat naturally occurring microbial inhibitors have been recovered from a wide variety of plants including herbs & spices, many of those antimicrobial contribute to the food stuffs natural resistance to deterioration. At present *Centella asiatica* is used for dementias and cognitive disorders, in the treatment of chronic venous insufficiency, diabetic microangiopathy and atherosclerotic plaques and in dermatological pathologies such as wound healing and scars prevention of striae gravidarum and psoriasis.

2. Material and Methods

2.1 Plant material

The plant material used was the leaves of *Centella asiatica*

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collected from herbal garden of Himachal Institute of Pharmacy, Paonta Sahib (HP) and identified by Botanical Survey of India, Dehradun.

2.2 Plant extract

The plant leaves were dried under shade, reduced to moderately coarse powder and macerated with 50% ethanol (w/w) at room temperature for 15 days. After filtration, the solvent was removed under reduced pressure (yield 5.75%).

2.3 Test Animals

The toxicity study as carried out using female and male Swiss mice (25-35 g, b.w.). Animals were kept at ambient temperature 25 ± 2 ℃ with 55% - 65% relative humidity and a 12h light dark cycle. They had free access to water and normal laboratory diet (Lipton India Ltd.).

2.4 Acute toxicity study

The animals were divided in to one control group and five treated groups, each group consisting of six animals for statistical validation. The control group received saline and each treated group received the acetone extract in dose of 100, 500, 1000, 2000 and 4000 mg/kg through gastric intubation. These doses were 10-100 times higher than effective doses in other studies. The animals were observed continuously for 3 hrs and then they were observed each hour during 24 h after administering the extract to observe any change in general behavior or other physiological activities as per OECD guidelines (6). At the end of the experiment animals were sacrificed by cervical displacement.

2.5 Subacute toxicity study

The animals were divided in to one control group and four treated groups, each group consisting of six animals for statistical validation. The control group received saline and each treated group received the acetone extract in dose of 500, 1000, 2000 and 4000 mg/kg through gastric intubation for 15 days (once a day in the morning). The animals were weighed each 3 days. At the end of the experiment, blood was collected from the orbital sinus under ether anesthesia for biochemical and hematological analysis. After the blood collection, the animals were sacrificed by cervical displacement and selected organs (liver, heart, spleen, left kidney and left lung) were removed for macroscopic analysis.

The Biochemical parameters evaluated included creatinine, calcium, inorganic phosphorous, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed using commercial kits. The hematology parameters were determined for the control and 1000, 4000 mg/kg groups and included red blood-cell count, hematocrit and leukocyte count.

2.6 Statistical analysis

The data are presented as Mean ± S.E. and the statistical significance between the groups was analyzed by means of an analysis of variance followed by Tukey's multiple comparison tests. The minimum level of significance was set at $P \leqslant 0.05$.

3. Results

Oral administration of the acetone extract of *Centella asiatica* in a dose from 100 to 1000 mg/kg did not produce significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses and gastrointestinal effect in male and female mice. The treatment with the extract did not decrease the water and food consumption. These effects are observed during the experimental period (24 h). During the experimental period no deaths occurred in any of the groups. These results showed that in single dose, there is no adverse effect of *Centella asiatica* indicating that the medium lethal dose (LD50) is higher than 4000 mg/kg for male and female mice. The body weight of the animals treated with acetone extract once a day during 15 days (subacute treatment) did not shows any significant change when compared with control group. The macroscopic analysis of the target organs of the treated animals (liver, lungs, heart, spleen and left kidney) did not shows significant changes in colour and texture when compared with control group. The results of the organ weight are summarized in Table 1.

During the experimental period, there were no treatment-related effects on the hematological parameters evaluated in Table 2.

Estimations of the serum activity such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium and inorganic phosphorous did not showed any significant changes however alterations in the serum concentration of creatinine were observed (Table 3).

Mean values of six animals ± S.D. *P<0.05; **P<0.01 vs. control group (Tukey's test). Control group received saline. No significant difference was observed in any parameter, except in liver (2000 and 4000 mg/kg).

Table 1

Effect of oral administration of *Centella asiatica* extract on body and organ weight.

Dose mg/kg	Control	500	1000	2000	4000
Body (g)	33.5±3.13	32.2±1.80	35.0±3.70	31.0±3.2	32.2±2.3
Liver (g)	1.649±0.202	1.410±0.138	1.462±0.221	1.30±0.174*	1.16±0.172**
Heart (g)	0.142±0.012	0.12±0.015	0.160±0.035	0.152±0.024	0.11±0.026
Kidney (g)	0.162±0.018	0.154±0.014	0.172±0.022	0.168±0.025	0.12±0.0232
Spleen (g)	0.182±0.090	0.152±0.029	0.162±0.049	0.122±0.011	0.12±0.0235

Table 2

Hematological parameters after 15 days treatment with the *Centella asiatica* extract.

Parameter	Control	1000 mg/kg	4000 mg/kg
Red blood cell (mm3)	9.040±0.368	8.092±0.448	8.78±0.125
Hematocrit (%)	45.8±4.60	43.8±1.520	47.045±2.60
Leukocyte (x 106/mL)	7.548±2.190	8.728±2.489	7.10±1.685

Table 3

Effect of treatment with the *Centella asiatica* extract on Biochemical parameters

Dose (mg/kg)	Control	500	1000	2000	4000
ALT (U/L)	101.9±20.10	103.9±6.5	81.90±15.80	88.20±25.70	80.45±10.20
AST (U/L)	48.60±8.10	43.50±8.50	45.30±8.70	46.80±8.50	50.60±11.40
Calcium (mg/dL)	6.480±0.450	6.970±0.85	6.745±0.318	7.564±0.378	7.280±0.0350
Creatinine (mg/dL)	0.248±0.048	0.250±0.01	0.149±0.02**	0.209±0.03	0.238±0.020
Inorganic phosphorous (mg/dL)	5.950±0.540	5.810±0.60	5.970±0.635	5.920±1.158	5.936±0.668

Values are mean ±S.D. **P<0.01 vs. control group (Tukey's test).

4. Discussion

Herbal medicines are used throughout in developed and developing countries and represent a substantial proportion of the global drug market. It is therefore essential to established internationally recognized guidelines for assessing safety, efficacy and quality. The world health assembly –in resolutions has emphasized the need to ensure the safety and quality of herbal medicines as per existing national legislation and national and regional norms. *Centella asiatica* is frequently used for the treatment of various ailments. The importance of this plant in folk medicine as well as its promising pharmacological properties verified in our laboratories, make studies about its toxicity very important. Abdulla et al., 2010 (7), carried out an acute toxicity study of ethanolic extract of *Centella asiatica* at a dose of 2 and 5 g/kg and the animals were kept under observation for 14 days. All the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. This is similar to the present study which showed that the acetone extract of *Centella asiatica* is safe in oral administration in rodents. The doses used in this study were 10–100 times higher than those used in other experimental pharmacological studies. Regarding to the acute oral toxicity study of Chitrala Roopesh et al., 2011 (8), for the ethanolic and methanolic extracts of *Centella asiatica* the mortality was not observed up to 5000 mg/kg body weight. Jayashree G et al., 2003 (9), was observed that the oral treatment with 50 mg / kg / day of crude methanol extract of *Centella asiatica* for 14 days significantly increased the anti-oxidant enzymes, like Superoxide Dismutase (SOD), Catalase and Glutathione Peroxidase (GSHPx), which is also beneficial for body defense mechanism against various diseases. Similarly our study demonstrated that *Centella asiatica* seems to be destitute of toxic effects, which could be compromise the medicinal use of this plant in folk medicine. However, further studies are under progress to confirm this evidence.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to Dr. Gaurav Gupta, Director, Himachal Group of Institutions and Principal, Himachal Institute of Pharmacy, Paonta Sahib, HP, for providing necessary research facilities.

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