ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 11, pp.75711-75715, November, 2018

DOI: https://doi.org/10.24941/ijcr.33436.11.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EVALUATION OF *CONVOLVULUS PLURICALIS*WHOLE PLANTS EXTRACT FOR ITS ACUTE ORAL TOXICITY IN *ALBINO* RATS

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ARTICLE INFO

Article History:
Received 10th August, 2018
Received in revised form
19th September, 2018
Accepted 22nd October, 2018
Published online 30th November, 2018

Key Words:

Convolvulus pluricalis, Acute Oral Toxicity, OECD Guidelines 425, Median Lethal Dose (LD_{50}), and Neuro-suppressant.

ABSTRACT

Background: *Convolvulus pluricalis* an herbal drug is known medicinal plant used extensively in the Indian system of medicine. In this regard to this several experiments has been conducted to know the LD_{50} and the actual effective (therapeutic) dose, but the results are inconsistent. Thus the acute oral toxicity study was carried out to find out the median lethal dose of the Hydro-Alcoholic extract whole plant extract of *Convolvulus pluricalis,* which will be used for further experiment to know its effective dose and the clinical trials. **Objectives:** The present study is carried out to evaluate the Hydro-alcoholic whole plant extract of *Convolvulus pluricalis* (HACP) for its acute oral toxicity in *Albino* rats. **Methods:** Acute oral toxicity of Hydro-Alcoholic whole plant extract of *Convolvulus pluricalis* was assessed in *Albino* rats with three doses of the extract at 175, 550 and 2000mg/kg bwt. The mortality, clinical signs & the body weight of the rats were recorded in 0 (prior to administration), 7th& 14th days. At the end of the observation period rats were sacrificed and studied for any histological changes in brain, heart, liver and kidney tissues. **The results:** Administration of HACP to the rats for 175, 550 &2000mg/kg bwt, were normal up to 1 hour and later on showed a sedative effect for the following 4 hours. Decreased motor activity was observed only in 550 &2000mg/kg bwt of HACP administered rats. All the animals appeared normal from day one to throughout the experimental procedure. No significant changes were seen in the histological structure of the brain, liver, kidney & heart. **Conclusion:** HACP signified as Nero- suppressant, the drug can be used in the treatment of neurological disorders characterized by hyperactivity of the neurons. The present data could provide adequate confirmation of the safety of CP for further experimental studies on a standardized formulation of the whole plant extract.

***Citation: Dr. Gangadhara Swamy, Dr. Suresh R Rao and Dr. Rajendra Holla,* 2018.** "Evaluation of *convolvulus pluricalis*whole plants extract for its acute oral toxicity in *albino* rats", *International Journal of Current Research*, 10, (11), 75711-75715.

INTRODUCTION

Human beings have always depended on the nature for his food, clothing, shelter, and medicine to cure different varieties of diseases for their good healthy life (Agarwal, 2013). But as the sciences advance a new medicinal system called modern system of medicine came to an existence where the chemical are isolated from plant origin or new chemical formulation was synthesized. But in the last few decades, it is noticed from the clinicians that there were a lot of side effects of the synthetic medicine, so that scientist are now focusing more on the herbal medicines to replace them. Since then there has been a potential development in the field of herbal medicine, and these herbal medicinal plants are gaining acceptance across the world because of their less side effects (Fatima, 2012).

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The Ayurveda pharmacopeia of India consists of the whole plant of Convolvulus pluricaulis (CP) (Shankhpushpi in Sanskrit) is one such effective herb which has been used since ancient times by physicians because of its pharmacological and healing properties. The plant contains alkaloid, volatile oil, flavonoids, phytosterol, carbohydrates, ceryl alcohol, and scopoletin (Singh, 2000). Dietary feeding of this plant has been found to increase protein synthesis in the hippocampus, thus enhancing memory and learning in experimental animals (Sinha, 1989). The plant is mainly used as a rasayana, which is advocated for use in rejuvenation therapy. CP has been found to augment both cognitive function and memory-enhancing effects in many behavioral studies (Singh, 1977). The chloroform extraction of this plant has potent insect anti-feed ant constituents like 20-oxodotriacontanol, tetratriacontanoic acid, and 29-oxodotriacontanol (Bhakuni, 1996). Various dosage forms and a wide variety of derived products have been used in the Indian system of medicine and also reported for its therapeutic activity experimentally and clinically in numerous

scientific journals. Ayurvedic Pharmacopoeia of India has stated that all parts of CP have medicinal properties (Anonymous Ministry of Health and Family Welfare, 2001). *Shankhapushpi* is considered as *Medhya Rasayana* (memory enhancer) in *Ayurvedic* literatures (Acharya, 2002) and has been used as Rejuvenator, anti‑ageing, mental stimulant and tranquilizer (Yoganarasimhan, 2000), formulations containing CP also used as an immuno‑enhancer (Acharya, 2002). The juice of the whole plant possessed anti-ulcerogenic effect and is comparable to sucralfate and also prevents menorrhagia. The grounded fine powered paste of the plant is used to treat abscesses. Whole plant ethanol extract of CP when administered to cholesterol fed mouse, after 90 days there is a significant reduction in serum cholesterol. Studies have also shown that the root extract of the CP had regulated hyperthyroidism in female mice (Dhingra, 2007).

The interest in the behavioral effect of HACP from the previous publication started only from the last two decades. Most of the reports are on rodents, a large majority of them using laboratory mice. Earlier animal studies using HACP did not report on any adverse effect on behavioral and histological changes of rodents. However, later studies have demonstrated changes in the growth and behavior in different mouse and rat strains. These studies vary in their dose, duration of exposure and strain of mice/rats. AlokNahata *et al*. stated that administration of the ethyl extract of CP to rats and subjected to the open field exploratory behavior test did not show any significant increase/decrease of locomotor activity. But at doses of 200 mg/kg bwt significantly reduced the neuromuscular coordination (Nahata, 2009). As per the OECD guidelines before the clinical trial on humans for any drug, it should be tested on animals to define its LD_{50} values and its effective/therapeutic dose. The name LD_{50} is an abbreviation for "Median Lethal Dose 50%". It is the quantity of the substance involved (usually per body weight) to kill 50% of the trial population. LD_{50} is the amount of a drug, given oral all at once, which causes the death in more than 50% (one half) of experimental animals in a group (http://www.oecd.org/oecd/ pages/home/display general/0, 3380, EN-document-524-nodirectorate-no-24-6775-8, FF. Html). One of the ways to know the LD_{50} is the short-term poisoning potential (acute toxicity study) of that material. For any toxicological experiment routinely used animals are rats and mice. It is commonlyuttered as the quantity of chemical administered (e.g.mg) per 100 gm (for smaller creatures) or per kg (for bigger creatures) of the body weight of the test animal.The LD_{50} can be found for any route of entry or administration, but dermal and oral administration methods are the most common (Jothy *et al*., 2011; Sabeeha Shafi, 2013). In spite of several researches being already covered during the past decades on the dynamic ingredients and the toxicity of CP, only scattered information is accessible and there is need to re‑collect it. Therefore, it is not possible to arrive at any conclusion regarding the lethal dose of CP and more data have to be acquired. With this view the present work was planned to study the CP whole plant extract for its acute oral toxicity in *Albino* rats.

MATERIALS AND METHODS

Plant material

The whole plants of CP were collected from the Sri Dharmastala Ayurveda medical college and research center, Udupi, Karnataka, India. The plant material was stored in ambient conditions for further survey.

Preparation of extracts

The CP whole plants were dried in the shade and powdered in our research lab with the help of pulverize. The HACP was prepared by soaking 500gms of powdered whole plants of the CP in 2 liters of 50% ethanol and 50% cold distilled water for 24 hours, filtered and concentrated by evaporating on water bath till free from water. The extract has been stored in an airtight container under normal temperature (Manyam *et al*., 2004).

Experimental animals

This study was performed in a CPCSEA approved laboratory under registration number 115/1999/CPCSEA following all ethical practices as laid down in the guidelines for animal care. This study has been approved by the Institutional Animal Ethics Committee with the reference no. KSHEMA/ IAEC/08/2017. The study procedure described in this study meets the requirements of the OECD guidelines for testing of chemicals, number 425, "Acute Oral Toxicity-Acute Toxic Class Method". Female Albino rats (9-11 weeks), weighing between 200 - 250 g were used. All animals were handled according to the guiding principles given by the Council for International Organization of Medical Sciences (CIOMS) on animal experimentation. Animals were supplied by the Department of Pharmacology, K.S. Hegde Medical Academy, Deralakatte, Mangalore. Rats were maintained under standard laboratory conditions, with a constant 12 hour light/dark cycle and controlled temperature (25 ± 2 °C) with access to drinking water and pellet diet ad libitum (Pritchett-Corning *et al*., 2009; Howard-Jones, 1985).

Chemicals

The solvents and chemicals required - CMC as a suspending agent & Distilled water as a solvent.

Methodology

Female Albino rats were selected based on their days of acclimatization. According to the OECD - 425 guidelines, the rats were randomly divided into 3 groups, namely Group A, Group B & Group C, with each group having 6 rats (n=6). They were kept fasting for overnight (but with the free access to water). On test day 0, the rats in each group had received a single dose of the HACP by oral gavage method dosages at 175, 550, 2000 mg/kg bwt respectively. Approximately after 17 hours of fasting, but with free access to water, the animals were continued with proper diet (El Hilaly *et al*., 2004; Meenatchisundaram, 2010).

Calculation for the preparation of the stock solution:

Group A: 175 mg of CP is mixed with 50 mg of CMC & the mixture is dissolved in 10 ml distilled water to get the concentration (conc.) of 17.5 mg/ml.

Group B: 550 mg of CP is mixed with 50 mg of CMC & the mixture is dissolved in 10 ml distilled water to get the conc. of 55 mg/ml.

Group C: 2000 mg of CP is mixed with 50 mg of CMC & the mixture is dissolved in 10ml distilled water to get the conc. of 200 mg/ml.

Procedure

The animals were observed for the acclimatization before the oral gavage of the drug and also daily during the test period for clinical signs such as, sedative effect, decreased locomotor activity, breathlessness & mortality/viability and were recorded during the first 30 minutes and at approximately with the duration of. 1, 2, 3 & 4 hours after administration of test drug on day 0 and twice daily during days 1-14 (Sabeeha Shafi, 2013). Body weights of all the rats were recorded on test day 0 (prior to administration), and also on test days 7 & 14. At the end of the 14th day of the observation period, the animals were deeply anesthetized with ether.

All the animals were observed for any gross/ macroscopic pathological changes and Brain, Liver, Heart, and kidneys from the representative groups of animals were removed and processed for the histological studies as follows (Underwood, 2013). The animals were deeply anesthetized with ether and fixed on a dissection board and its chest cavity was opened to expose the heart. About 15 ml of 0.9% saline was perfused through the left ventricle at the rate of 1 ml/min. This was followed by perfusion with 10% formalin, about 250 ml/adult rats, at the same rate. The animals were decapitated and 5-6 mm thick coronal section of a brain with the cerebral cortex, midbrain, liver, kidneys, and heart was removed and kept in 10% formalin for 24 hours (Post-fixation). Paraffin blocks were prepared as given below (Hopwood, 1996).

Flow chart of the procedure

- Fixation: By transcardial perfusion
- Dehydration:
  - 70% alcohol- 2 hours
  - 90% alcohol- 2 hours
  - Three changes in absolute alcohol 2 hours
- Xylene 2 hours
- Three changes in paraffin wax for 1 hour each
- Embedding in freshly filtered paraffin wax.
- Sections of 3-4 microns thickness for the brain tissue and 5 microns thickness of the remaining tissues were cut, using a rotary microtome. 5-6 sections were selected and mounted on air-dried gelatinized slides.
- Brain sections were stained with 0.1% cresyl violet and the remaining tissues were stained with the Hematoxylin & Eosin stains.

The sections were stained as follows

- Xylene - 1 min.
- Descending grades of alcohol (100%, 90%, and 70%) for 2 min each
- Distilled water - 5 min.
- 0.1% Cresyl violet stains at 60° C and H & E stain at room temperature for 30 min.
- Cool at room temperature
- Distilled water - 5 min.
- Ascending grades of alcohol (70%, 80%, 90%, and 100%) for 1-2 min each
- Xylene - 1/ 2min
- Mount with DPX

Each section of the tissue was observed under the light microscope with 10X and 40X for the microscopic characteristics and which was compared with its normal tissue.

Statistical analysis

Statistical analysis is performed by using Student's T-test, One way ANOVA where ever it is applicable by using Graph Padinstat software.

RESULTS

Oral administration of HACP of three different doses for 14 days in Albino rats did not produce any significant toxicity symptoms in rats, including the highest dose tested at 2000 mg/kg bwt. Rats in the group A were completely normal in their activities for the 1st hour and later on showed a sedative effect for the following 4 hours on day 0 and found normal on the next 14 observation days (Table A1). Rats in the group B were completely normal in their activities in the initial two hours and later on showed the sedative effect and decreased motor activities for the following 4 hours on day 0, and found to be normal on the next 14 observation days (Table B1). Rats in the group C were completely normal in their activities for the 1st hour and later on showed the sedative effect and decreased motor activities for the following 4 hours on day 0, and found to be normal on the next 14 observation days (Table C1). It was also noticed that by the end of the experimental procedure, there was a marginal increase in the body weight in rats with the dose of 175mg/kg weight, which was not significant. A significant increase in the body weight in rats with the doses at 550 and 2000mg/kg bwt was noticed (Table D).

DISCUSSION

Convolvulus Pluricalis is one of the plants used in the treatment of the various diseases. Whole plant extract of CP possesses anti-ulcerogenic, anti-diabetic, anti-cancer and anti-oxidant, reduces serum cholesterol, regulated hyperthyroidism, immuno-enhancer, Rejuvenator, anti-aging, mental stimulant, and tranquilizer, helps in increase in cognitive function and memory-enhancing properties (Agarwal *et al*., 2014). The literature review of phytochemical, bioactivity and toxicity of CP whole plant extract has been documented. However, very few studies related to the level of toxicity of CP whole plants have been conducted but not consistent in their reports. Therefore, our present study has been conducted to determine the level of acute toxicity of HACP whole plants on Albino rats for 14 days. In our present study oral administration of 2000 mg/ Kg body weight of the CP whole plant extract did not show any mortality. Similar types of results have been obtained from previous studies conducted by SabeehaShafi *et al*. (2013). A study conducted by Pawar SA in 2001, the lethal dose in mice with Convolvulus microphyllus (very close plant with the same family and species) by oral administration was found to be 1250 (1000-1400) mg/kgbwt. The sedative effect of CP in mice was observed at doses greater than 200 mg/kgbwt, and moderate to a marked decrease in locomotor activity which lasted 1-2 h. At a higher dose (more than 1 g/kgbwt) animals died due to respiratory distress (Pawar *et al*., 2001). Results obtained from our studies indicate that after oral administration

Table A1.

Animal ID no.	Test days																	
	0*					1	2	3	4	5	6	7	8	9	10	11	12	13	14
	0.5	1	2	3	4														
A1	N	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N
A2	N	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N
A3	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N
A4	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N
A5	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N
A6	N	S	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

S=Sedative effect, M=Decreased locomotor activity, B= Breathlessness, D= Death

**Examinations were performed within the first 0.5 h and at approximately 1, 2, 3 and 4 h after treatment on test day 0*

Table B1.

Animal ID no.	Test days																	
	0*					1	2	3	4	5	6	7	8	9	10	11	12	13	14
	0.5	1	2	3	4														
B1	N	S	S	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
B2	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N
B3	S	S	S	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
B4	S	S	S	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
B5	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N
B6	N	S	M	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N

S=Sedative effect, M=Decreased locomotor activity, B= Breathlessness, D= Death

**Examinations were performed within the first 0.5 h and at approximately 1, 2, 3 and 4 h after treatment on test day 0*

Table C1.

Animal ID no.	Test days																	
	0*					1	2	3	4	5	6	7	8	9	10	11	12	13	14
	0.5	1	2	3	4														
C1	N	S	S	M	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N
C2	N	S	S	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
C3	S	S	S	M	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N
C4	S	S	M	M	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N
C5	S	S	M	M	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N
C6	N	N	M	M	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N

S=Sedative effect, M=Decreased locomotor activity, B= Breathlessness, D= Death

**Examinations were performed within the first 0.5 h and at approximately 1, 2, 3 and 4 h after treatment on test day 0*

Table D

Groups (mg/kg bwt)	No. of Animals (n)	0 day	7^{th} day	14^{th} day
175	6	222.22± 1.458	226.72±3.469	233.90±3.420
550	6	206.63±1.456	213.70±1.530	219.05±1.390***
2000	6	206.82±1.061	213.30±0.7559	218.25±0.6103***

*Values are expressed as mean ± SE. *P values were considered significant using one-way ANOVA. Anovasignificane F= 2.550 for 175mg/kg bwt. ***=P<0.001, Anova Significance F= 18.201 to 550 mg/kg bwt and ***=P<0.001, Anova Significance F= 47.644 for 2000 mg/kg bwt.*

of the HACP at 2000mg/kg bwt the rats appeared dull for a brief period (on test day 0) after which they became normal throughout the experimental procedure up to 14 days. The temporary dullness on test day 0 may be due to the possible role of the Neuro-suppressive effect of the extract. Based on the results obtained from our experiments, it indicates that HACP administered orally can be used safely as a drug in the treatment of neurological disorders characterized by the hyperactivity of neurons. The above experiment has shown that even at 2000 mg/kg bwt of HACP did not have any mortality, Moreover; it was also observed that all the rats had gained body weight by the end of the experimental procedure.

Conclusion

HACP was assessed for its acute oral toxicity in the rats. It was found that by oral administration of HACP at 2000mg/kg bwt was non-toxic. Rats treated with the plant extract at dose 550 & 2000mg/kg bwt and above, shown a sedative effect and moderate to marked decrease in locomotor activity which lasted 1-4 h.A brief period of dullness at low dosage indicates a possible role of the extract in Neuro-suppression as well. Thus, it can be used as a drug in the treatment of neurological disorders characterized by the hyperactivity of neurons. So the results of this study collectively specify that oral administration of CP is not associated with any toxicologically significant effects, and the data could provide satisfactory preclinical evidence of safety to launch a clinical trial.

Conflict Of Interest: The authors do not have any conflict of interest on publication of this manuscript.

Acknowledgement

We sincerely thank to: Sri Darmastala Ayurveda Medical College & Research Centre, Udupi, Karnataka, India for providing plant material for this study. The Management of Subbaiah Institute of Medical Sciences and Research Center Shivamogga. The Management of K.S. Hegde Medical Academy, Deralakatte, Mangalore.

Author Contributions

The first author designed and worked on the experiment, the second author collected the review of literature and also planned for the experiment and the third author helped in planning and statistical analysis of the work. All the three authors equally contributed to the overall study.

Source Of Funding: Self-funding

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