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Research Article

EVALUATION OF THE ACUTE ORAL TOXICITY EFFECTS OF COLEUS FORSKOHLII ROOT EXTRACT (COLEGEX®) IN ALBINO WISTAR RATS

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ABSTRACT

In accordance with the general assumption that oral use of herbal products is not harmful, there is not much data available about their potential for toxicity. This makes it difficult to assess the safety of herbal extracts used as food and medicine. In India, Sri Lanka, Thailand, Nepal, and other countries with subtropical climates, Coleus forskohlii, a member of the Lamiaceae family, flourishes as an aromatic plant. Since the plant is administered orally, only limited information is known about its safety. The current study's objective was to evaluate the acute oral toxicity of C. forskohlii root extract (Colegex®) in female albino Wistar rats. The OECD Guideline 423 protocols were used to conduct acute oral toxicity research on C. forskohlii (Colegex®). To ascertain the median lethal dose over a 14-day period, C. forskohlii (Colegex®) was administered orally once daily to the three rats in the treatment group at a dose of 2000 mg/kg bw. Even though the limit dose was kept at 2000 mg/kg bw throughout the 14-day study period, C. forskohlii (Colegex®) did not cause any mortality. Throughout the time frame of the experiment, all of the animals appeared healthy. A single oral dose of 2000 mg/kg bw of C. forskohlii (Colegex®) did not result in any clinical signs or death in rats examined over a 14-day period. More than 2000 mg/kg bw is determined as the median lethal dose for C. forskohlii (Colegex®).

INTRODUCTION

In recent years, people have started taking more dietary supplements, particularly those with herbal constituents. Herbal components are frequently used as a part of the diet for traditional medical purposes and are known to be safe when used in dietary supplements. But their safety is still an issue when they are taken in concentrated forms, such as tablets and capsules. Recent research has shown that herbal products, particularly those used for weight loss, can have adverse effects (AEs), such as severe liver damage[1-3] and gastrointestinal stress[4,5]. Ethnomedicinal herbs have been utilized for centuries in developed as well as developing countries as therapeutic treatments for the treatment, mitigation, and prevention of diseases in both humans and animals[6]. The prevailing consensus is that medicinal herbs are considered safe for use in therapeutic applications because they are all-natural goods free of synthetic preservatives. More than 85% of human and animal disease issues, from bacterial infections to cancer and immunological diseases, are treated using natural products[7]. People in developing countries are using more herbal medicines because they think that since they are natural, they are generally safe for treating diseases. In addition, it is a misconception that herbal medicines and natural products affect the body less than synthetic drugs[8]. Although the herbal remedies are thought to be safe, they occasionally contain heavy metals, pollutants, contaminants, and combinations of several harmful chemicals[9,10]. There is a possibility of several types of interactions occurring after the injection of a specific chemical agent into a biological system that may have negative effects, frequently leading to death. To evaluate the safety of developing pharmaceuticals and herbal substances, toxicological studies have become necessary for these reasons. The primary resource for the regulatory safety assessment of natural herbal products is published data on adverse reactions and toxicity[11]. Pharmaceutical companies conduct a number of toxicity studies, including acute, subacute, and chronic toxicity testing, as part of the evaluation of the safety of new drugs. Acute oral toxicity testing is frequently done as a preliminary step to screen substances for safety and evaluate their toxicity[12].

The Indian Native *Coleus forskohlii* Briq. (Lamiaceae) is an aromatic herb. Over the past 40 years, medical researchers have focused a lot of attention on the herb (Figure 1) since it is the only substantial plant source of forskolin, a bioactive diterpene molecule with a variety of pharmacological advantages. Since ancient times, plants of the Coleus species have been utilized as

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herbal medicines to treat a variety of conditions affecting the digestive, respiratory, circulatory, and nervous systems[13]. In India, pickles or condiments made from the roots of this folk remedy, *C. forskohlii* (Makandi), have a long history of use. The plant's root paste is used externally on tumours and boils by people in northern India. The tribal people of Trichigadi (Kotas) use the decoction of the roots as a tonic in south India. In 1974, researchers found forskolin, an active phytochemical in *C. forskohlii* (CF), which has a wide range of biological effects.

Fig 1 *C. forskohlii* plant with root

Because of its forskolin content, the *C. forskohlii* plant is used to prevent cancer metastases, where an absence of activated cyclic adenosine monophosphate (cAMP) can be a major factor in the development of the disease[14]. The additional pharmacological properties of forskolin[15] are also connected to its capacity to directly activate the adenylate cyclase enzyme, resulting in increased amounts of cAMP from adenosine-5′-triphosphate (ATP). There haven't been sufficient comprehensive studies of the toxicity of *C. forskohlii* (Colegex®) roots described in the literature, though. Evaluating the acute oral toxicity of *C. forskohlii* (Colegex®) roots was the objective of the current study in order to boost public confidence in their ability to treat a variety of illnesses in humans.

MATERIALS AND METHOD

Preparation of *C. forskohlii* extract

C. forskohlii (Colegex®) is a standardized extract manufactured and registered by Ingex Botanicals Pvt. Ltd., Nelamangala, Bangalore, Karnataka, India.

Experimental animals

Healthy female Wistar albino rats, aged 8–12 weeks, were obtained from Radiant Research Services Pvt. Ltd. in Karnataka, India. The procedures for regulating and supervising animal research investigations were followed as per CPCSEA Registration Number 1803/PO/RcBi/S/20 15/CPCSEA. Each animal has been determined using picric acid and given a unique number. The animal was kept in a standard stainless steel cage with access to regular food and water in bottles. Aqua Guard's service provided free access to water. Animals have always had access to uncontaminated, clean drinking water. They were kept in these cages under normal laboratory settings, including a cycle of 12 hours of light and 12 hours of darkness, a temperature of 22±3°C, and a relative humidity range of 50–70%. Each procedure involving animals was conducted in an ethical manner under the supervision of trained professionals. Before the study started, the research protocol was reviewed and approved by Radiant Research Services Pvt. Ltd.'s Institutional Animal Ethical Committee (IAEC).

TOXICOLOGICAL EVALUATION

Acute oral toxicity

Three animals received a single dose of *C. forskohlii* (Colegex®) by oral administration of 2000 mg/kg bw after being fasted over night with free access to water. After almost 4.0 hours of adhering to dosing, food was once again provided. 10 ml/kg bw was used for administration. Individual animal observations were made daily for the following 14 days, with special focus on the first 4 hours and at least once every 30 minutes over the first 24 hours. In order to record any symptoms, signs, or behavioral abnormalities, all the rats were watched at least twice per day. Six animals were used in the limit test, which was carried out at 2000 mg/kg bw and monitored for 14 days. The liver, heart, spleen, and kidneys were carefully removed and weighed. And it was removed for histopathology analysis. Further analysis was done on the body weight and gross pathological examinations. The clinical symptoms and signs of the drugs were assessed in order to determine a safer dosage.

STATISTICAL ANALYSIS

Data were expressed as mean ± SEM for data analysis.

RESULTS AND DISCUSSION

Acute oral Toxicity

The current investigation, which was carried out in accordance with OECD Guidelines 423, showed that *C. forskohlii* (Colegex®) did not cause any mortality over the 14-day trial period, even when the limit dose was kept at 2000 mg/kg bw. A single oral dose of 2000 mg/kg bw of *C. forskohlii* (Colegex®) did not result in any of the clinical signs or deaths observed in rats over a 14-day period (Table 1). According to the current investigation, which was carried out in accordance with OECD Standard 423, *C. forskohlii* (Colegex®) did not cause any deaths over the 14-day study period, even though the maximum dose was kept at 2000 mg/kg bw. Throughout the experiment, each animal appeared to be in normal condition (Table 2). After the dose was administered, behavioral changes were carefully monitored. Throughout the course of the trial, none of the animals showed any aberrant symptoms. All surviving animals had gained body weight by the 3rd, 7th, and 14th days as compared to day 0 (Table 3, Figures 2 and 3). All the animals were shown to have gained weight during the trial, which is common in healthy animals. Finally, all living animals were slaughtered and eliminated after the gross and microscopic pathological abnormalities had been noticed and documented (Table 4 and Fig 4). No tissues or organs were preserved. In rats examined over a 14-day period, *C. forskohlii* (Colegex®) at a single oral dose of 2000 mg/kg bw did not result in mortality or clinical signs. More than 2000 mg/kg bw is the median lethal dose of *C. forskohlii* (Colegex®).

Table 1 Clinical and Behavioral Signs/Symptoms

CODES	OBSERVATIONS	SIGNS/SYMPTOMS
1	NAD	02-40 codes observations are not seen
2	Accidental death	---
3	Partial Cannibalism	An animal of a species consuming part of another animal of the same species
4	Total Cannibalism	An animal of a species consuming the major organs of another animal of the same species
5	Dead	Irreversible cessation of all body functions, manifested by absence of spontaneous breathing and total loss of cardiovascular and cerebral functions
6	Moribund condition	Approaching death animal will not be available for examination for next day
7	Weakness	A weak bodily state as expressed by difficulty in rising, a shuffling, disinclination to move, eating slowly and a drooping posture
8	Lethargy	A level of consciousness characterized by decreased interaction with objects in the environment, sluggishness, abnormal drowsiness
9	Salivation	Flow of saliva, Drooling (Abnormally abundant flow of saliva)
10	Lacrimation	Flow of tears
11	Discharge	Abnormal discharge
12	Snuffling (Unusual respiratory pattern)	A bubbling sound from the nasal cavities
13	Bronchial rales	An abnormal respiratory sound (crackles) in auscultation of lungs
14	Cough	A forceful release of air from the lungs
15	Dyspnea (Unusual respiratory pattern)	Shortness of breath
16	Corneal opacity	Opaque white spot on the cornea
17	Cataract	Opacity of the crystalline lens of the eye
18	Diarrhea	Diarrhea is the frequent passage of loose, watery, soft Stools.
19	Hematuria	Presence of blood in the urine
20	Piloerection	Erection of hair
21	Response to handling	Normal response to approach.
22	Convulsions	Violent involuntary contraction of a muscle or muscles
23	Repetitive Circling	Continuous circling
24	Head tilted on one side	Head facing towards some other direction other than straight
25	Ataxia	Inability to control voluntary muscle movement
26	Dermatitis	Inflammation of the skin
27	Blister	A local swelling of the skin that contains watery fluid
28	Urticaria	An itchy skin eruption characterized by weal's with pale interiors and well-defined red margins
29	Necrosis	Death of a portion of tissue differentially affected by local injury
30	Erythema	Redness of the skin
31	Oedema	A swelling from effusion of watery fluid in the cellular tissue beneath the skin or mucous membrane
32	Cyanosis	Bluish discoloration of the skin and mucous membranes
33	Paralysis	Loss of sensation over a region of the body
34	Edema	An excessive accumulation of serous fluid in tissue spaces or a body cavity
35	Crepitation	A dry, crackling sound or sensation
36	Dehydration	Loss of water and salts. The skin turns pale and cold, the mucous membranes lining lose their natural moisture
37	Dull	Lacking responsiveness or alertness
38	Posture	Position of the body or of body parts
39	Epistaxis	Bleeding from the nose
40	Urine dribbling	Leaking of urine

The minimal adverse reactions of herbal medications have influenced people to believe for centuries that they are both safe and effective. This assumption might have had a significant impact on the rural population's widespread and indiscriminate usage of these formulations. These formulations are frequently given over a long period of time without sufficient dosage monitoring by professionals or knowledge of the potential harmful effects of such prolonged use[16]. Therefore, scientific

understanding of oral toxicity is crucial in order to both determine future doses and expose potential clinical symptoms induced by drugs under investigation. Because the general population considers widely used herbal remedies to be safe and given high dosages of plant extracts or chemicals, but these changes are quickly reversible[18]. Animal body weights changes, whether positive or negative, are invariably indicators

Table 2 Clinical signs and Behavioral observation during the study in rats

Sl. No.	Parameter	Complete nest (n=20)	Incomplete nest (n=10)	P-value
1	Weight (gm)	60.5±16.6	18±3.4	1.33^{NS}
2	Total length (cm)	54.5±10.8	34.1±5.9	6.84^{NS}
3	Total width (cm)	15.65±0.8	14±1.9	0.0026**
4	Stalk length (cm)	22.3±6.4	16.6±4.5	0.0185**
5	Nest length (cm)	17.95±2.6	17.8±2.09	0.8771^{NS}
6	Entrance tube length (cm)	14±6.5	-	1^{NS}
7	Stalk width (cm)	4.3±1.12	5.2±1.47	0.0737^{NS}
8	Entrance tube width (cm)	8.8±1.6	-	1^{NS}
9	Egg chamber circumference (cm)	38.65±8.7	34.5±3.8	0.00082**
10	Entrance tube circumference (cm)	19.1±4.9	-	1^{NS}
11	Threshold (cm)	2.15±0.5	2.1±0.21	0.5750^{NS}

*Observation of first four hours after treatment; 01 – No Abnormality Detected (02-40 codes observations are not seen)

Table 3 Body weight of rats during the study period of *C. forskohlii* (Colegex®)

Animal ID	Dose	Treatment			
		Before		After	
		Day 0	Day 3	Day 7	Day 14
RA 01					
RA 02	2000mg/kg bw	188.3±2.33	191.0±2.31	195.3±2.60	205.3±3.18
RA 03					
RA 04					
RA 05					
RA 06	Limit test				
	(2000mg/kg bw)	189.3±0.84	193.5±0.76	197.8±0.83	207.3±0.95
RA 07					
RA 08					
RA 09					

Table 4 Result of gross pathological examinations in *C. forskohlii* (Colegex®)

Animal ID. No	Dose	Macroscopic lesions
RA 01		No macroscopic alteration occurred
RA 02	2000mg/kg bw	No macroscopic alteration occurred
RA 03		No macroscopic alteration occurred
RA 04		No macroscopic alteration occurred
RA 05		No macroscopic alteration occurred
RA 06	Limit test (2000mg/kg bw)	No macroscopic alteration occurred
RA 07		No macroscopic alteration occurred
RA 08		No macroscopic alteration occurred
RA 09		No macroscopic alteration occurred

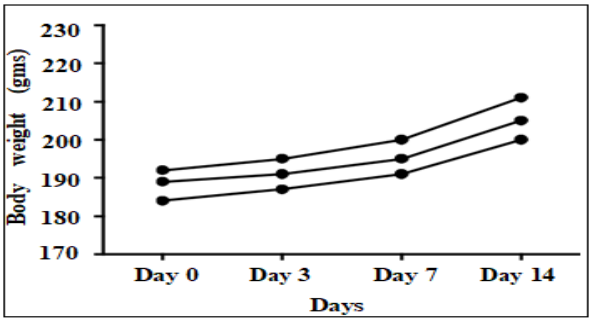

Fig 2 Body weight of animals at 2000 mg/kg bw dose from Day 0 to Day 14

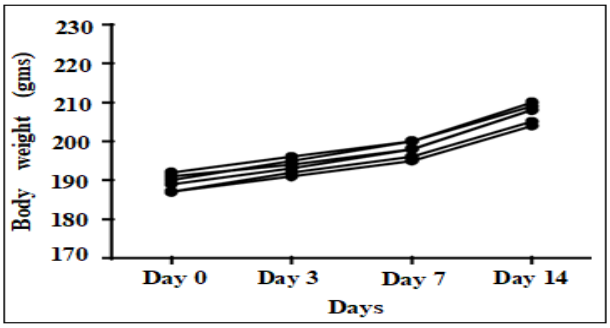

Fig 3 Body weight of animals at limit test dose (2000 mg/kg bw) from Day 0 to Day 14

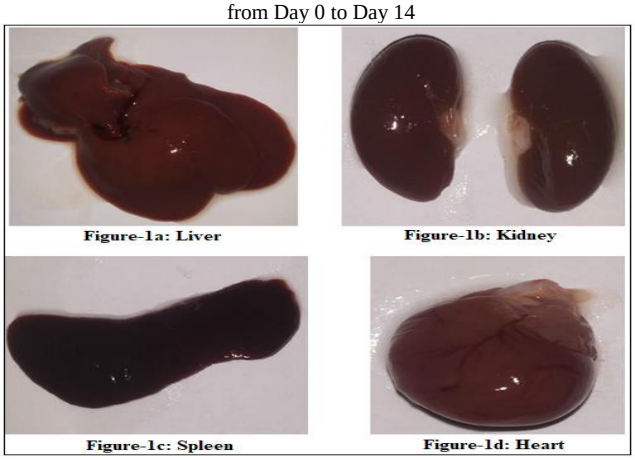

Fig 4 Gross pathological examination of treated animals (Macroscopic observation)

without any side effects that could compromise health, they are frequently used for self-medication[17]. Animals may exhibit small behavioural alterations as a result of metabolism when of the negative effects of medications and chemicals[19]. However, body fat accumulation, rather than the harmful

effects of medications or chemicals, is more closely linked to a rise in animal body weight[20].

OECD 423 criteria were followed in the current study, which was carried out in rats. In the investigation on acute toxicity, rats received an oral dose of 2000 mg/kg bw and were then individually monitored for the first four hours, then during the next 24 hours, and finally once daily for 14 days. There were no aberrant indications or symptoms after the administration of *C. forskohlii* (Colegex®), and there were no fatal infections or large weight gains. Gross and macroscopic examination of the animal tissues after post-mortem revealed no abnormalities. The conclusion is that *C. forskohlii* (Colegex®) can be safely used in pharmaceutical formulations for medicinal purposes.
During the course of the experiment, general behavior, harmful consequences, and mortality were observed. Body weights were noted on test days 0 (before administration), 3, 7, and 14. All of the animals underwent necropsies and macroscopical examinations. By day 14, compared to day 0, all of the animals that survived had gained weight. When the animals were sacrificed, necropsies were performed, but no abnormalities were found. The rats examined during the observation period did not exhibit any indicators of toxicity or mortality at the limit dose of 2000 mg/kg bw. According to the findings, Category 5 is assigned to *C. forskohlii* (Colegex®), whose median lethal dose in female rats following a single oral administration is above 2000 mg/kg bw.

CONCLUSION

These results allow us to reach the conclusion that *C. forskohlii* (Colegex®) is not harmful at the single oral dose of 2000 mg/kg bw examined here and did not result in any overt symptoms during the acute oral toxicity studies. The kidneys, liver, spleen, and hearts of the rats in the control and treatment groups did not exhibit any significant abnormalities, according to the histological analysis. Additionally, data from experiments on the acute oral toxicity of *C. forskohlii* (Colegex®) were gathered to boost confidence in its safety for use in developing medications for humans. The acute oral LD50 for *C. forskohlii* (Colegex®) has been calculated to be 2000 mg/kg bw, making it effective for therapeutic use in pharmaceutical formulations.

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