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ORIGINAL ARTICLE

Acute oral toxicity and *in-vitro* antioxidant activity of aqueous extract of *Croton sparsiflorus*

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ABSTRACT
The aim of the study was to investigate the in-vivo acute toxicity and in-vitro antioxidant activity of aqueous extract of Croton sparsiflorus (AECS). The acute toxicity test was conducted in Wistar albino rats. AECS was administrated as the single dose at different concentrations 200, 400, 600, 800, 1000 and 1200 mg per kg and observed for behavioral changes and mortality rate. In this study, no visible behavioral changes and mortality rates were observed up to 1200 mg/kg of the Wistar albino rats. The antioxidant activity of AECS was evaluated by DPPH free radicals scavenging assay. Ascorbic acid was used as reference compound. AECS exhibited 36.8% of free radical scavenging activity as compared with ascorbic acid. The present study concludes that AECS did not exhibit any toxic effects on mice activities and also this extract possesses significant antioxidant property. Therefore, AECS may be useful in therapeutic purposes.
KEY WORDS: *Croton sparsiflorus, acute toxicity, antioxidant activity, albino rats.*

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INTRODUCTION

According to an evaluation, 80% of the world population in developing countries relies on traditional plant medicine [1]. In the last decade, there has been an exponential development in the use of herbal products for the treatment of different types of diseases [2]. The treatment involving herbal drugs were practiced in India for a long time. Herbal drugs are generally considered as safe and do not have side effects. However, herbal drugs can be toxic to humans. It is therefore important to understand and validate the toxicity of herbal compounds for therapeutic use. Oxidative stress take place, as soon as there is extreme free radical production and low antioxidant defense, which leads to chemical modification of biomolecules, causing structural and functional disruptions [3]. Oxidative damage plays a major pathological role in human diseases such as arthritis, cancer, diabetes, and inflammation [4]. Currently available synthetic antioxidants showed low solubility, negative health effect and moderate antioxidant activity [5]. Recently, there has been an upsurge of interest in the therapeutic potential of medicinal plants, like antioxidants in reducing free radical induced tissue injury. Many plant species have been investigated for novel antioxidants[6] but there is still demand to find more effective antioxidants from plant species. DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a stable radical of organic nitrogen characterized by its deep purple color and a maximum absorbance in the range of 515-520 nm. The DPPH method was first reported by, Blois 1957 [7]. who observed that the DPPH radical was reduced by the thiol-containing amino acid cysteine and/or other compounds[7]. In the presence of hydrogen atom, (free radical scavenging antioxidant) the absorption intensity is decreased and the solution is discolored according to the number of electrons captured [8]. The present study focuses on both the acute oral toxicity on Wistar albino rats and *in-vitro* antioxidant activity of the medicinal plant *C. sparsiflorus.* The *Croton* species was introduced to India during the late 1890s from Paraguay. In 50 years, its supply became countrywide. In these decades, the compounds derived from *Croton* species comprise to cure human ailments reaffirm its medicinal value.[9] The extracts of *C. sparsiflorus* have previously been reported to help in therapeutic cuts and/or open wounds and external treatment for ringworm infection [10].

Although, a number of reports on the medicinal benefits of the aqueous extract of *C. sparsiflorus* Sare available, the *in-vivo* toxicological effect of the plant extract has not been reported. It is consequently deemed necessary to evaluate the acute oral toxicity of the *C. sparsiflorus* in Wistar albino rat model. This

toxicity study would serve as an important baseline for further studies in developing this plant as herbal medicine.

MATERIALS AND METHODS

Plant collection

The plant material was collected from papanasam surroundings of Thanjavur district. The plant was identified as *Croton sparsiflorus* by Professor Dr. M. Jegadeesan professor and Head, Department of Herbal Science, Faculty of Science, Tamil University, Thanjavur using a voucher specimen (TUH-92).The latex was obtained from fresh leaves and diluted with water at 1:1 ratio and centrifuged at 10,000 ×g for 10 min to have aqueous soluble extract. This extract is referred to as aqueous extract, which was used for all analysis.

Chemicals

DPPH was purchased from Sigma- Aldrich, USA. Ascorbic acid, methanol and all other solvents and chemicals were purchased from Hi Media Pvt. Ltd., unless stated otherwise.

Animal models

The experiments were carried out on 6-8 weeks old healthy Wistar albino rats, both male and female weighing between 120 and 150 g. The experimental procedures followed in this study were according to internationally acknowledged principles on laboratory animal use and care. The experimental procedures relating to the animals were duly approved by the University Animal Ethical Committee. Ethical clearance was obtained from the Institutional Animal Ethical Committee [PRIST/IAEC/PhD/CRD/03/2013-2014]. All procedures were in strict compliance with relevant laws, the Animal Welfare Act, Public Health Services document and guidelines established by the Institutional Animal Ethical Committee of the University.

Determination of acute toxicity

Adult male and female Wistar albino rats were kept in the departmental facility in well aerated polycarbonate cages under standard conditions (30±2°C, 12-h light/dark cycle). To carry out the toxicity study on Wistar albino rats, permission was obtained from the Institutional Animal Ethical Committee. Experiments were carried out complying with the rules of the Committee. The mice were housed in cages at random selected ones were tagged and marked on the cages for identification. They were permitted to adapt laboratory conditions for a week before starting the experiment. Drinking water and food were provided *ad-libitum* during the experiment period. The acute oral toxicity of AECS was evaluated in rats according to the procedures reviewed by the Organization for Economic Co-operation and Development (OECD). The dose of 200 to 1200 mg per kg of AECS was administrated to rats in the treatment groups by oral route. The dose limits were selected on the basis of acute oral toxicity studies in rats, in conformity with the Organization in support of Economic Co-operation and Development (OECD) guidelines 425.[11] The acute toxicity test was carried out in mice by giving doses of 200, 400, 600, 800, 1000, 1200 mg per kg body weight. Food was provided to the rats approximately an hour after treatment. The rats were observed in detail for any sign of toxicity effect within the first 3 h, after the treatment period and up to a period of 14 days. Visual observations on mortality, behavioral pattern, and changes in physical appearance, wound, ache and signs of illness were evaluated up to 14 days at 24 h interval [12].

Determination of free radical scavenging activity

The *in-vitro* antioxidant activity of AECS was carried out to determine the free radical scavenging capacity using DPPH method. One hundred microliter (100 µl) of sample was added to 2.8 ml methanol and 100 µl of 0.1 % methanolic DPPH. The control was prepared by adding 100 µl of 0.1 % methanolic DPPH to 2.9 ml of methanol. The suspension was incubated for 30 min in dark condition. The absorption of the solution was measured at 517 nm (UV-vis. Spectrophotometer-Systronics). The antioxidant activity of the sample was calculated with known standard of ascorbic acid at 1.0 mg/ml. Percentage of DPPH scavenging activities of the extract and reference standard were determined according to the method of George *et al*., [13].

RESULTS AND DISCUSSION

The main objective of this study was to understand the acute oral toxicity and *in-vitro* antioxidant activity of aqueous extract of *C. sparsiflorus* (AECS). This experiment was conducted on Wister albino rats for 14 days. A dose level of 0 mg – 1200 mg per kg of mice was used to determine the acute oral toxicity (Table 1). This study revealed that no mortality was observed until 14 days, suggesting the non-toxic nature of the AECS. The body weight was also found to be normal and there were no significant changes in body weight of the mice, irrespective of the dose level. Various other physiological characteristic of rats were also determined during the experimental period and found that the rats have no symptoms of any adverse effects (Table 2). The body weight, food and water intakes were found to be unaltered during the

treatment period when compared to control group. Therefore, the results concluded that AECS had no toxic effect on Wister albino rats.

Table 1. Effect of aqueous extract of *C. sparsiflorus* on Wister albino rats

Extract [a] (mg/kg of rats)	Dead rats (after 24 h)	Dead rats (after 14 days)	Body weight (g) (at 0 h)	Body weight (g) (after 24 h)	Body weight (g) (after 14 days)
0	0	0	145.23	145.25	146.12
200	0	0	152.62	152.67	156.21
400	0	0	120.40	120.38	125.43
600	0	0	124.62	124.59	128.23
800	0	0	149.62	149.65	154.36
1000	0	0	155.35	155.38	158.55
1200	0	0	154.32	154.29	157.41

[a] *C. sparsiflorus* aqueous extract was prepared by suspending the latex in de-mineralized water at 1:1 ratio. The suspension was centrifuged at 10,000 ×g for 10 min. The aqueous soluble portion of the extract was collected and air dried. The powdered aqueous extract was re suspended in de-mineralized water and administered in rats, proportionate to its body weight.

Table 2. Effect of aqueous extract of *C. sparsiflorus* on physiological characteristics of Wister *albino rats.*

Characteristic	0 h		3 h		Day-1		Day-7		Day-14	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Skin and fur	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N
Lethargy	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N
Mortality	N	N	N	N	N	N	N	N	N	N

Test, aqueous extract of *C. sparsiflorus* administered rats,
N, Normal.

Table 3. Effect of aqueous extract of *C. sparsiflorus* on free radical scavenging activity

Aqueous extract of *C. sparsiflorus* (µg/ml)	Time (min.)	Ascorbic acid (scavenging activity in %) [a]	*C. sparsiflorus* (scavenging activity in %)
50	5	37.98	32.09
100	10	50.21	33.65
200	15	60.08	34.25
300	20	67.38	35.21
400	25	74.67	36.05
500	30	84.54	36.77

[a] Ascorbic acid was used at 1.0 mg/ml.

DPPH method is widely used to evaluate the free radical scavenging activity of plant extracts and compounds [14]. DPPH is free radicals which change violet to yellow color when DPPH is getting reduced by hydrogen or electron donation from the samples. Those samples having the ability to reduce the DPPH are considered as the potent antioxidant.[15] The present study; we investigated the ability of the AECS to act as donors of hydrogen atoms or electrons in the transformation of DPPH radical into its reduced form. The results indicated that DPPH gets reduced 36.8% and 84.54% by AECS and ascorbic acid respectively (Table 3). Although AECS showed less free radical scavenging than the ascorbic acid, which was considered as the reference standard.[16] Overall results suggested that *C. sparsiflorus* did not exhibit toxic effects in mice and had significant free radicals scavenging activity which may be very useful in therapeutic applications.

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