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Evaluation of Skeletal Muscle Relaxant Activity of Ethanolic Extract of the Leaves of *Costus pictus* D. Don in Swiss Albino Mice



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ABSTRACT

Skeletal muscle relaxants are the agents that are used to treat both muscle spasm and spasticity, acting both as antispasmodic and antispasticity agents but it has serious side effects. *Costus pictus* D. Don is traditionally known to treat various diseases such as diabetes and anxiety due to its medicinal properties. Anxiety is accompanied by skeletal muscle contraction. Hence an attempt at calming down or relaxing contracted skeletal muscles in anxiety conditions was made. The aim was to evaluate the skeletal muscle relaxant activity of the Ethanol extract of leaves of *Costus pictus* D. Don in comparison with diazepam. The ethanol extract was given in Swiss Albino Mice at a dose of 100 mg/kg body weight and 200 mg/kg body weight. Skeletal muscle relaxant activity was assessed by using Rota-rod apparatus and Inclined screen test. The results are promising for further investigation of efficient skeletal muscle relaxant activity.



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INTRODUCTION:

A skeletal muscle relaxant such as diazepam is a drug which affects skeletal muscle function and decreases the muscle tone. It may be used in alleviating symptoms such as muscle spasms, pain, and hyperreflexia. They are Benzodiazepine class of drugs which are centrally acting.^[1]

The side effects of antispasmodic agents and antispasticity agents cause them to be used with caution. Previous reports have shown that 10-20% of adults suffer from insomnia. Mainly affect CNS, evident in the form of sedation, drowsiness, ataxia, impaired judgment. In increased doses can cause depression of CVS and respiratory system.^[2]

The present study was conducted to evaluate the skeletal muscle relaxant activity of this plant.

Costus pictus D. Don formerly known as insulin plant or Painted Spiral Ginger. It is a species of herbaceous plant in **Costaceae** family native to Mexico, traditionally known in India due to its Anti-diabetic property. It has nice narrow long leaves with characteristic wavy edges. It can be recognized by its yellow flowers with red spots and stripes, stem with spiral leaves and light airy and tissue paper like flowers. Red painted stem enhances the beauty of *Costus pictus D. Don*. Phytochemical investigations reveal the presence of chemical constituents such as carbohydrates, triterpenoids, proteins, alkaloid, tannins, saponins, flavonoids, steroid, and appreciable amounts of trace elements.^[3] This work was an attempt to explore Skeletal Muscle Relaxant activity of an Ethanolic extract of the leaves of *Costus pictus D. Don* in Swiss albino mice since it was found to relax contracted muscles in anxiety conditions.

MATERIALS AND METHODS:

Plant Material -

Fresh *C. pictus* leaves were collected from the Medicinal garden of Oriental College of Pharmacy, Navi Mumbai. The fresh plant specimen was identified and authenticated at Blatter Herbarium, St. Xavier's College, Mumbai. Leaves were washed, shade dried and powdered.

Preparation of Extract

The powdered samples were extracted with ethanol by soxhlet apparatus for a defined period with continuous agitation. The extracts were then filtered, condensed and stored for further studies. [4][5]

Preliminary phytochemicals screening

The Rotarod phytochemical test of the ethanolic extract of dried leaves of *Costus pictus D.Don* was performed for the presence of various active principles (tannins, saponins, flavonoids, quinines, glycosides, cardiac glycosides, triterpenoids, phenol, alkaloids, steroids, oils and fats, phytosterols) using standard procedures. [4][5]

Animal

Animals required for research (studying acute toxicity and skeletal muscle relaxant activity respectively) were approved from IAEC of Oriental College of Pharmacy, Sanpada, Navi Mumbai 400 705. IAEC Proposal Number: OCP/IAEC/2016-2017/02. The animals used for the study were procured from Bombay Veterinary College, Parel, Mumbai 400 012. Animals procured were specifically female Swiss Albino mice weighing 20-25 gm. The Animals were allowed to acclimatize for a period of 7 days, housed under standard conditions of temperature ($25\pm 2^{\circ}\text{C}$) and relative humidity (30%–70%) with a 12:12 light-dark cycle, fed with pellet diet and water.

Drugs and Chemicals

The Diazepam as the free sample which was the leftover stocks of the college that were used as the standard skeletal muscle relaxant drug. Distilled water was used as the vehicle.

Acute Toxicity Study

The acute toxicity of ethanolic extract of *Costus pictus D.Don* was determined in female Swiss albino mice. Animal was fasted overnight prior to the experiment

The method followed to perform the AOT was the Acute Toxic Class method i.e. OECD – 423.

1. Three doses were chosen from the Annex II of OECD 423 i.e. Minimum, Medium and

Maximum i.e. 50 mg, 300 mg, and 2000mg after sighting study.

2. Total of 9 animals was chosen i.e. 3 animals per group and three groups were taken namely) *Costus pictus D. Don* Extract - 50mg, 300mg, 2000 mg
3. A single dose was administered and animals were observed for a period of 14 days for clinical signs and mortality.^[6]

Selection of Dose for Pharmacological Screening.

The ethanolic extract was found to be nontoxic up to a dose of 2000 mg/kg and did not cause death, therefore, it was considered to be safe. Hence, one-tenth of this dose, that is, 200 mg/kg body weight and half of the one-tenth dose, that is, 100 mg/kg, were used for the elucidation of skeletal muscle relaxant activity. The extracts were suspended in the vehicle in such concentrations so as to administer 100 and 200 mg/kg doses to mice through oral route. All drugs were freshly prepared before each experiment. DPZ (5mg/kg; p.o) used as the standard.

Experimental Design

The animals were divided into four groups of six mice each for each activity. The drugs were administered as shown below:

1. Group I - Normal control (0.5ml of Distilled water).
2. Group II - standard (0.5ml of diazepam 5mg/kg).
3. Group III - 0.5ml of 100mg/kg extract of *Costus pictus D. Don*.
4. Group IV - 0.5ml of 200mg/kg extract of *Costus pictus D. Don*.

SCREENING OF SKELETAL MUSCLE RELAXANT ACTIVITY:

MODEL 1- INCLINED SCREEN TEST

Procedure –

The inclined plane test is to determine the skeletal muscle relaxant activity. The Plane consisted of transparent glass which was left on an inclined at 30 °C. The mice that tried to

move out of the plane glass without sliding off, were used for the test. The investigation was made at 15-30 min intervals, subsequent to the administration of control, standard and extract. The mice were kept in the superior part of the inclined plane and were given 30 sec to hang on or to fall off.^[7]

MODEL 2 – ROTA ROD TEST

Procedure –

Rota rod apparatus consisted of a base platform and an iron rod of 3 cm diameter and 30 cm length, with a non-slippery surface rotating at a speed of 32 rpm. This rod was divided into three equal sections by two disks, thus enabling three mice to walk on the rod at the same speed. Intervals between the mounting of the animal on the rod and falling off of it were recorded as the performance time. After the administration of the standard drug and extract, the performance time was measured at 15-min time intervals for 90 min for 300 sec (cut-off time).^[8]

Statistical analysis

The values were expressed as (n=6) mean \pm SEM. The results were subjected to statistical analysis by using **one way** and **two-way** analysis of variance (ANOVA) followed by Turkey's multiple comparisons test using Graph Pad Prism 7.

RESULTS AND DISCUSSION:

Table 1 – Preliminary Phytochemical screening of Ethanolic extract of leaves of *Costus pictus D. Don.*

Phytochemicals	Ethanolic Extract
Tannins	+ve
Saponins	+ve
Flavonoids	+++ve
Quinones	+ve
Glycosides	+ve
Cardiac glycosides	+ve
Triterpenoids	+ve
Phenol	+ve
Alkaloids	+++ve
Steroids	+ve
Oil and fats	+ve
Phytosterols	++ve

Acute Toxicity Study

During acute toxicity studies, the extract produced no adverse effects at dose 2000 mg/kg and did not cause any death up to a dose of 2000 mg/kg in mice.^[6]

Skeletal muscle relaxant activity

MODEL 1- INCLINED SCREEN TEST

Table 2: Skeletal Muscle relaxant activity by using inclined screen test in mice

Group	Dose	Time taken to slide off the screen (sec)
Normal	0.5 ml Distilled water	123.667 ± 1.498
Standard	5mg/kg Diazepam	6.167 ± 0.477
Extract Test -1	100 mg Low Dose	18.000 ± 0.365****
Extract Test -2	200 mg High Dose	15.667 ± 0.422****

Values are expressed as (Mean ± SEM), n= 6..Statistically analyzed by **one- way** analysis of variance (ANOVA) followed by Turkeys Multiple Comparison Test.****P<0.0001 Vs standard

MODEL 2 – ROTA ROD TEST

Table 3: Skeletal Muscle relaxant activity by using Rotarod test in mice (Recording of fall off time from rota rod apparatus)

Group	Dose	Before	After (fall off time in seconds)				
		0 mins	15 mins	30 mins	45 mins	60 mins	90 mins
Normal	0.5ml	166.833	166.833 ±	170.167 ±	169.000 ±	149.333	144.000±
	Distilled water	± 3.701	3.701	4.556	7.983	± 8.024	13.289
Standard	5mg/kg	150.667	68.333	60.167 ±	54.333±	47.500 ±	39.167±
	Diazepam	± 6.716	±3.442	3.664	3.293	7.356	4.301
Extract Test -1	100mg Low Dose	147.000	118.000 ±	105.000±	119.500 ±	120.333 ±	122.833 ±
		± 9.420	1.932****	6.314****	4.829****	4.432****	8.542****
Extract Test -2	200mg High Dose	142.833	98.333 ±	98.000	102.833±	98.500±5.4	107.500 ±
		± 4.339	2.728**	±3.396***	4.362****	82****	5.506****

Values are expressed as (Mean ± SEM), n= 6.Statistically analyzed by **two- way** analysis of variance (ANOVA) followed by Turkeys Multiple Comparison Test..****P<0.0001 Vs standard.

Table 4: Skeletal Muscle relaxant activity by using Rota rod test in mice (% activity)

Group	Dose	15 mins	30 mins	45 mins	60 mins	90 mins
Standard	5mg/kg	54.64 %	60.06 %	63.93 %	68.47 %	74 %
	Diazepam					
Extract Test -1	100 mg Low Dose	19.72 %	28.57 %	18.70 %	18.14 %	16.44 %
Extract Test -2	200 mg High Dose	31.15 %	31.38 %	28 %	31.03 %	24.73 %

Values are expressed as (Mean ± SEM), n= 6. Statistically analyzed by **two- way** analysis of variance (ANOVA) followed by Turkeys Multiple Comparison Test.

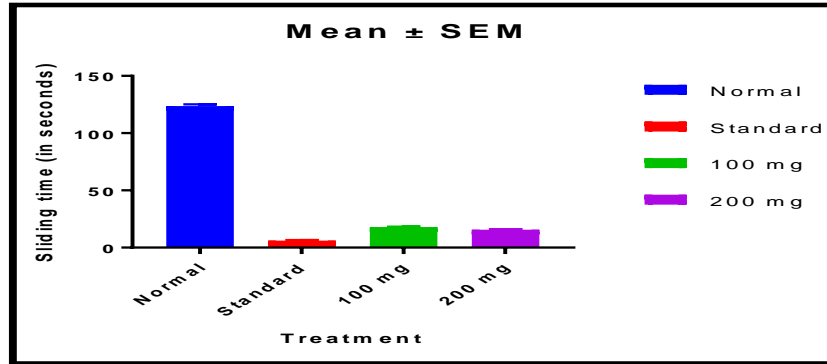


Fig.1: Graph showing an effect of an Ethanolic extract of leaves of *Costus pictus D. Don* on inclined screen test) in mice. Values are expressed as (Mean ± SEM), n= 6.

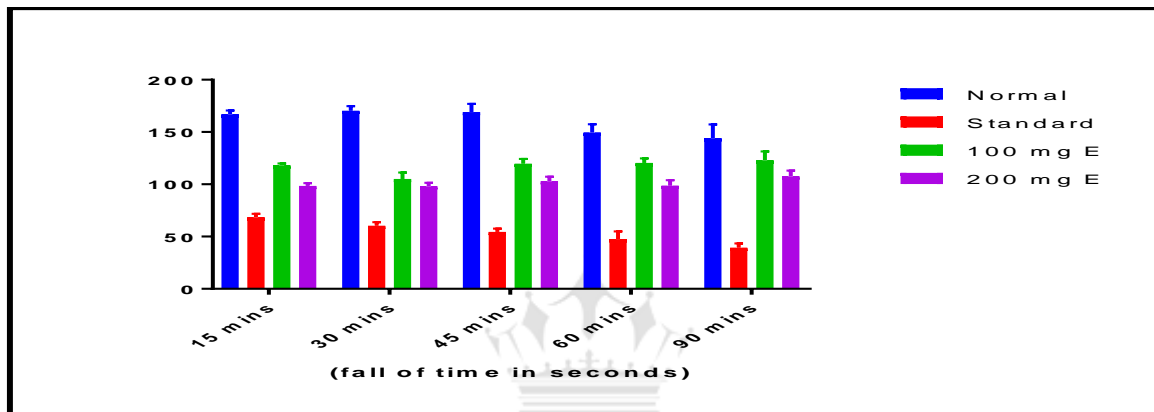


Fig.2: Graph showing an effect of an Ethanolic extract of leaves of *Costus pictus D. Don* on Rotarod test (Recording of fall off time) in mice. Values are expressed as (Mean ± SEM), n= 6.

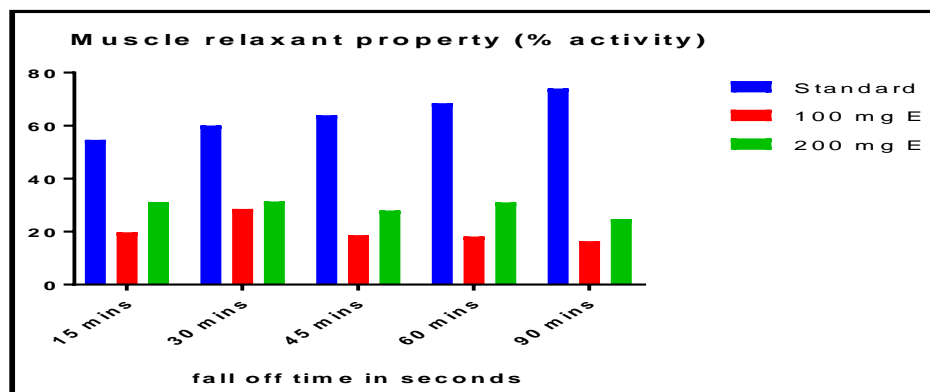


Fig. 3: Graph showing an effect of an Ethanolic extract of leaves of *Costus pictus D. Don* on Rota rod test (% activity) in mice. Values are expressed as (Mean ± SEM), n= 6

In the present study, the test samples of the Ethanolic extract of leaves of *Costus pictus D. Don* were tested for skeletal muscle relaxant activity. In this study, fall off time and muscle grip was taken as a measure of skeletal muscle relaxant activity. It was observed that both the doses showed significant results. From the p values and the statistical analysis, the test – 2 (200mg/kg dose) was found to be most effective when compared to test -1. (100mg/kg dose) in both the models. Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plants containing flavonoids possess activity against many CNS and muscle disorders.^[9]

Phytochemical tests of *Costus pictus D. Don* revealed the presence of flavonoids (quercetin) and hydrocinnamic acid derivative (sinapic acid) present which is known to reduce anxiety.^{[9][10][11][12][13]} As Anxiety is accompanied by muscle contraction.^[14] Hence an attempt at calming down or relaxing contracted skeletal muscles in anxiety conditions was made. It may be possible that the mechanism of skeletal muscle relaxant action of *Costus pictus D. Don* could be due to the binding of any of these phytochemicals to the GABAA-BZD complex. So the skeletal muscle relaxant activity of *Costus pictus D. Don* might involve an action on GABAergic transmission.^{[15][16]}

CONCLUSION:

The present study sheds light on the potential role of *C. pictus* in ameliorating the clinical signs and symptoms by virtue of its skeletal muscle relaxant activity potential. The herb, besides being an effective traditional antidiabetic medicine in India may also play a major role in calming down contracted skeletal muscles. The study suggests that the ethanolic extract of *Costus pictus D. Don* leaves has skeletal muscle relaxant activity. As the comparison is done with centrally acting benzodiazepine group of drug diazepam, it is assumed that the skeletal muscle relaxant activity effects of *Costus pictus D. Don* could be due to the interaction of flavonoids of the plant with the GABA/benzodiazepine receptor complex in brain.^[16]

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