

## Skeletal Muscle Relaxant Activity of N-Butanol Fraction of Methanolic Extract of *Cleome gynandra* Leaf in 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. *Cleome gynandra* (Cleomaceae) is traditionally used for various diseases because of its medicinal properties. The aim was to evaluate the skeletal muscle relaxant activity of the N-butanol fraction of leaves of *Cleome gynandra* comparison with diazepam. The butanol fraction was given in Swiss Albino Mice at a dose of 50 mg/kg, 100 mg/kg, 200 mg/kg body weight. Skeletal muscle relaxant activity was assessed by using Rota-rod apparatus. The results are promising for muscle relaxation. In this test, BF CG (50 mg / kg, 100 mg / kg, and 200 mg / kg) showed highly significant reduction in the time spent by the animals on the revolving rod when compared to the control ( $P < 0.01$ ). The standard drug (Diazepam) also showed a highly significant effect when compared to the control ( $P < 0.01$ ). Three different doses of BF CG (50, 100, and 200 mg / kg p.o.) showed a dose-dependent increase in muscle relaxation, that is, 67.85, 85.26, and 92.22, Time spent on revolving rod in Rota rod apparatus  $32.03 \pm 0.22$ ,  $29.06 \pm 0.30$ ,  $16.82 \pm 0.33$ , respectively further investigation of efficient skeletal muscle relaxant activity. The study may help in the development of cheap, effective and safe skeletal muscle relaxant drugs.

**Keywords:** Actophotometer, *Cleome gynandra*, rota-rod, soxhlet's apparatus, muscle relaxant.

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### I. Introduction

In modern days antispasmodic agents like cyclobenzaparin are used to treat musculoskeletal conditions. Antispasticity agents like dantrolene are used to relieve muscle hyper tonicity. The side effects of antispasmodic agents and antispasticity agents cause them to be used with caution<sup>(1)</sup>. Previous reports have shown that 10-20% of adults suffer from insomnia<sup>(2)</sup>. The present study was conducted to evaluate the skeletal muscle relaxant activity of this plant. *Cleome gynandra* Linn. [syn.C.pentaphylla Linn, Gynandropsis pentaphylla DC., gynandra (Capparaceae) family is an erect glandular-pubescent annual herb, popularly used in the Ayurveda, Siddha, Folk and Tibetan systems of medicine. This wild leafy vegetable is indigenous to the tropical and pan tropical regions and plays an important role in agricultural and nutritional systems of these regions. In many cultures, the boiled leaves are regarded as medicinal meal for the treatment of various ailments. Bruised leaves are reported to be rubefacient, vesicant, antiseptic, anti-inflammatory and analgesic and hence used to treat local pains, neuralgia, rheumatism and scorpion-sting. Oral administration of a decoction or an infusion of the boiled leaves or the leaf-juice has been recorded to facilitate child birth, to relieve stomach pain, beneficial in constipation, thread-worm infection, conjunctivitis, oral ailments, convulsions and in certain bilious disorders. Earlier investigations on the leaves of the Egyptian taxon have afforded certain flavonoids, triterpenoid saponins, sterols and fatty acids, a triterpene from the whole plant, glucosinolates and a number of anti-tick essential oil constituents. Extracts of the leaves and certain isolated flavonoids have been reported to possess antibacterial, antifungal, antineoplastic and anti-arthritis properties and improved the levels of endogenous antioxidants and also modulated glucose metabolizing enzyme activity<sup>(3)</sup>. Dietary phytochemicals, especially the polyphenolic antioxidants such as the ubiquitous flavonoids<sup>(4)</sup>, polyunsaturated fatty acids, tocopherols, vitamin-C and various inorganic micronutrients have been the subject of extensive research for their potential benefits to reduce the risk of degenerative diseases such as cardiovascular disease, several types of cancer, inflammation and neurological and other age-related disorders. Being diet derived, these compounds are generally regarded as safe chemicals based on their long history of use in the diet and have been demonstrated to possess strong antioxidant activities in vitro. Though the screening of antioxidant and radical scavenging activities of the taxon have been reported earlier<sup>(5-6)</sup>, but an investigation of the amounts of the potentially beneficial antioxidants

available in the extracts of varying polarities of this leafy vegetable will also be of importance. *Cleome gynandra* Linn acknowledged for its medicinal properties so it's important to reveal other medicinal and phytochemical elements. The phytochemical compound contains polar and non-polar functional groups and therefore their solubility varies in different solvents. Many researchers have already carried out the preliminary phytochemical study of solubility of chemical constituents in their estimation process. In the present investigation, skeletal muscle relaxant activity was carried out.

**Taxonomic position of *Cleome gynandra* L. is as follows**

Kingdom: Plantae

Division: Angiosperms

Class: Dicotyledones

Order: Capparidales (Capparales)

Family: Cleomaceae

Genus: *Cleome*

Species: *Gynandra*

**Vernacular names in India<sup>(7)</sup>**

Sanskrit: Pasugandhi, Ajagandha

Assamese: Bhutmulla

Bengali: Hurhuria, Shulte

English: Dog Mustard

Gujarat: Talvani, Dhelitalavan

Hindi: Hulhul, Hurhur, Kavalia

Kannada: Naram bele Soppu, Nayeetulasi

Kashmiri: Gandi Buti

Malayalam: Atunari vela

Marathi: Tilvan, Bhatvan, Mabli, Tilavana, Tilvant

Oriya: Anasorisia, Anasorisa, Hulhulia

Punjabi: BugraTamil: Nal valai, Nal velai

Telugu: Vaminta, Vayinta.

## II. Materials And Methods

### Collection of the plant

The fresh plant *Cleome gynandra* Linn was collected from local area of Warangal and authenticated by the Dr. K.Raju , professor, Department of Botany; Kakatiya university warangal collection number 1003.

### Preparation of leaf extract

Leaves were dried for one week at room temperature (in shade). Dried leaves were grinded in a blender to fine particles. Hot Extraction method Crude leaf extract was prepared by Soxhlet extraction method. Range Three polar to non-polar solvents was selected for the extraction, namely Ethanol, chloroform, petroleum ether, The 85 gm of dried finely grinded powder was uniformly packed into thimble and phytochemicals were extracted with 750 ml of three mentioned solvents separately. The extraction was carried out for 12 hours. Later extract was concentrated by keeping it on water bath for 50° to 60°C and stored at 0°C for further study.

### Animals

Swiss albino mice (SWR) aged six to seven weeks of either sex weighing about 25 – 30 g, were obtained from the Central Animal House. The animals were fed a Purina Chow diet, with water *ad libitum*, and were maintained under standard conditions of temperature, humidity, and light (12 hours light / 12 hours darkcycle). The experiment complied with the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethics Committee (IAEC); Registration number 285 / CPCSEA. The guidelines for the investigation of experiments in conscious animals were followed in all tests.

### Drugs and Chemicals

Diazepam (Lupin Laboratories Ltd., India), 10 mg / kg and normal saline (0.9% NaCl solution) were administered in a volume of 10 ml / kg. The extracts were suspended in distilled water and subjected to muscle relaxant activity using the Rotarod apparatus and Actophotometer. The extracts were administered orally (p. o.) in a volume of 10 ml / kg of body weight, in doses of 50 mg, 100 mg, and 200 mg / kg.

### **Acute Toxicity**

A total of 35 mice were randomly allotted to one control and six treatment groups. The animals were fasted overnight, prior to the experimental procedure. The method of up-and-down or staircase was used to determine the dose.<sup>(8-9)</sup> The procedure was followed as per the Organization for Economic Co-operation and Development (OECD) 423 guidelines. The extract in each case was administered orally in three doses: 0.5 g / kg, 1.0 g / kg, and 3 g / kg. The animals were observed for 24 hours for signs of toxicity and mortality.<sup>(10)</sup> In the acute toxicity tests, Cleome gynandra treated animals exhibited no alarming signs of toxicity. Only at the 3 g / kg dose level there was some decreased locomotor activity observed.

### **Selection of Dose for Pharmacological Screening**

The aqueous extract of Cleome gynandra was found to be non-toxic up to a dose of 2000 g / 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, as also half of this, that is, 50 mg / kg, were used for the elucidation of muscle relaxant activity.

### **Experimental Design**

Group I – Control Rats (Normal saline 10 ml / kg)

Group II – Standard (Diazepam 10 mg / kg)

Group III – BF CG 50 mg / kg

Group IV – BF CG 100 mg / kg

Group V – BF CG 200 mg / kg

**(I) Skeletal muscle relaxant activity (motorcoordination)**<sup>(11)</sup> The mice were divided into five groups consisting of six animals each. Group I served as the control, which received Normal saline 10 ml / kg, Group II received the standard drug Diazepam, at a dose of 10 mg / kg, p.o., Group III, IV, and V received the aqueous extract of Cleome gynandra orally at a dose of 50, 100, and 200 mg / kg. The animals remained on Rotarod (25 rpm) for five minutes or more after low successive trials were included in the study. After the administration of control, standard, and test material, the fall off time from the rotating rod was noted after 30 minutes. The difference in the fall off time from the rotating rod between the control and the treated mice was taken as an index of muscle relaxation.

**(II) Locomotor activity:** The spontaneous locomotor activity was assessed with the help of a photoactometer.<sup>(12)</sup> Each animal was observed for a period of five minutes in a square closed field arena (30 × 30 × 30 cm) equipped with six photocells in the outer wall. Interruptions of photocell beam (locomotor activity) were recorded by means of a six digits counter. To see the locomotor activity, the Actophotometer was turned on and each mouse was placed individually in the activity cage for five minutes. The basal activity score for all the animals was noted. Control normal saline, Standard Diazepam, and three different doses of aqueous extract of Cleome gynandra were given orally and after one hour of re-testing, and the activity score for five minutes was observed. The difference in the activity, before and after drug administration, was noted. The percentage decrease in motor activity was calculated.

### **Statistical Analysis**

The results were expressed as mean ± SD. Statistical analysis was carried out by using the Analysis of Variance (ANOVA) followed by Dunnet's multiple comparison tests using primer of windows McGraw– Hill software version 5.0.0.0 (2011). *P*-values < 0.05 were considered significant.

## **III. Results**

### **Rotarod Test**

For muscle relaxation In this test, BF CG (50 mg / kg, 100 mg / kg, and 200 mg / kg) showed highly significant reduction in the time spent by the animals on the revolving rod when compared to the control (*P* < 0.01). The standard drug (Diazepam) also showed a highly significant effect when compared to the control (*P* < 0.01). Low dose of BF CG (50 mg / kg) showed a significant effect (*P* value < 0.05) [Table 1]. Three different doses of BF CG (50, 100, and 200 mg / kg p.o.) showed a dose-dependent increase in muscle relaxation, that is, 67.85, 85.26, and 92.22, Time spent on revolving rod in Rotarod apparatus 32.03 ± 0.22, 29.06±0.30, 16.82± 0.33, respectively, when compared to the control. Maximum muscle relaxation was observed with 200mg / kg of Cleome gynandra. The result from the Rotarod test showed that the fraction significantly reduced the motor coordination of the tested animals.

### Actophotometer

Test for locomotor activity: The percentage of reduction in the locomotor activity with Diazepam (10mg / kg, p.o.) after 30 minutes was 89.5%, that is, there was a highly significant ( $P < 0.000$ ) decrease in locomotor activity compared to the control, whereas, three different doses of BF CG (50,100, and 200 mg / kg, p.o.) showed a dose-dependent decrease in the locomotor activity, that is, 67.85, 85.26, and 92.22, respectively, when compared to the control. Maximum muscle relaxation was observed with 200 mg / kg of Cleome gynandra. The values were highly significant ( $P < 0.000$ ) [Table 1].

**TABLE 1: Effect of Cleome gynandra butanol fraction on the locomotor activity on the actophotometer and muscle coordination on the rotarod apparatus**

Groups	Actophotometer score			Time spent on revolving rod in Rotarod apparatus (in seconds)
	5 minutes Before administration	60 minutes after administration	% of Reduction	
Group I (control) NS 10 ml / kg	178.4 ± 0.89	---	0	99.86 ± 0.56
Group II (standard) Diazepam 10 mg / kg	223.2 ± 0.55	8.84 ± 0.15***	98.25	15.33 ± 0.83**
Group-III BF CG 50 mg / kg	170.1 ± 0.758	31.6 ± 0.35***	67.85	32.03 ± 0.22*
Group-IV BF CG 100 mg / kg	199.8 ± 0.88	22.05 ± 0.55***	85.26	29.06 ± 0.30**
Group-V BF CG 200 mg / kg	189.1 ± 0.58	12.15 ± 0.54***	92.22	16.82 ± 0.33**

BF CG –Butanol fraction of Cleome gynandra. All values are Mean ± SD,  $n = 6$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.000$  when compared with the control

### IV. Discussion

In the present study the test samples of leaf extract of Cleome gynandra belongs to the family Cleomaceae were tested for skeletal muscle relaxant activity. Several reports are available on many plant species belonging to the same family exhibit skeletal muscle relaxant activity so the plant is presently studied<sup>(13-14)</sup>. In this study, fall off time and muscle grip were taken as a measure of skeletal muscle relaxant activity. Diazepam showed significant neuromuscular blocking action. In the present study test samples exhibited significant ( $P < 0.001$ ) skeletal muscle relaxant activity at a dose of 50mg/kg, 100 mg/kg and 200 mg/kg body weight. Among these test samples butanol fraction at dose of 200 mg/kg body weight exhibited more neuromuscular blocking action. It may be due to the presence of Phenols, carbohydrates, glycosides, saponins and flavonides.

### V. Conclusion

The result of our study shows a dose-dependent decrease in locomotor activity when compared to the control, on the Actophotometer. Maximum muscle relaxation was observed with 200 mg / kg of Cleome gynandra. The Rotarod test showed highly significant reduction in the time spent by the animals on the revolving rod when compared to the controls. The result from the Actophotometer test and Rotarod test showed that the extract significantly reduced the motor coordination of the tested animals. In conclusion, our data indicates that Butanol fraction of Cleome gynandra possesses sedative and skeletal muscle relaxant activities.

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