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## **Anxiolytic and CNS depressant effects of ethanolic extract of *Cleome brachycarpa* revealed after neuropharmacological screening**

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### **ABSTRACT**

Plant extracts have been used since ancient times for treatment of different diseases. They contain phytochemicals which possess multiple therapeutic effects. The aim of this study was to explore neuropharmacological effects of *Cleome brachycarpa*. Ethanolic extract of *Cleome brachycarpa* was diluted in DMSO and administered orally at 300mg/kg according to weight of animals. Various CNS screening tests have been performed on mice after acute dosing. The results showed decreased number of cage crosses, head dip, central square and peripheral square crossing, decreased struggling time in FST and increased time in light box in light and dark test. Thus it is concluded that *Cleome brachycarpa* possesses anxiolytic and CNS depressant effect after acute dosing.

**Key Words:** *Cleome brachycarpa*, Anxiolytic, CNS- depressant.



### **INTRODUCTION**

Since the beginning of human civilization, plants have been used for treatment of different ailments.

[1] Phytochemicals are synthesized by plants which are chemical compounds. Phytochemicals have biological significance includes antioxidants which may reduce risk of cancer, Alzheimer, aging and are beneficial in other diseases too.[2] Plants which contain phytochemicals when consumed by humans as a source of food are helpful in treating different diseases in human body. An integral part of traditional medicine is herbal medicine. Plants had been used for healthcare and medicinal purposes long before it was recorded in history. [3] *Cleome* belongs to a small family of flowering plants *Cleomaceae* which comprises of 9 genera containing more than 300 species. *Cleome* is the largest genus comprising of about 180-200 species. *Cleome* includes species which have ecological, ethno botanical and medicinal value. [4] It comprises of yearly growing herbaceous plants or perennial shrubs widely distributed in tropical and subtropical regions. Approximately 150 species of *Cleome* have been recorded in tropical regions thus showing its diversity in this region. [5] In India 15 species have been reported.[6]

It is a branched and spreading herb grown yearly with height of 3dm. It possesses racemose inflorescence that ends in leafy bracts. Petals are yellow. Stamens are 6 in number. Seeds are dark maroon-brown in colour, 0.5-0.8mm in diameter and slightly wrinkled. Flowering and fruiting season is from August to October. [7]

By spectroscopic analysis Cabralealactone, Ursolic acid and new triterpenoid dilactone deacetoxybrachycarpone has been isolated from *Cleome brachycarpa*. [8] The structure of cabralealactone, ursolic acid and deacetoxybrachycarpone was determined a year later. A new triterpenoid Cleocarpone was also isolated from *Cleome brachycarpa*. [9] Cleocarpone has double oxygen function at C-24 and belongs to dammarane series. Plant is used against abdominal discomfort. It possesses antibacterial and antifungal activity. The plant is used as appetizer and as carminative tonic. [10] It is used as anti-inflammatory agent. The leaves of plant are useful in leucoderma and are also considered useful in skin diseases caused by mites and scabies. It is also useful in rheumatism. [11] From literature it has been found that *Cleome brachycarpa* possesses anti-inflammatory, anti-bacterial and carminative effects however not much

work has been done regarding its effects on CNS. The aim of this study is to evaluate neuropharmacological profile of ethanolic extract of *Cleome brachycarpa* after acute dosing in order to gain knowledge regarding its efficacy and risks involved in its use.

## MATERIALS AND METHOD:

**Collection of plants:** The plant *Cleome brachycarpa* was provided by Dr. Iqbal Azhar Department of Pharmacognosy University of Karachi. The extract was obtained after drying, extracting and macerating with 95% ethanol.

**Animal's Selection:** For CNS parameters screening albino mice of either sex weighing 20-26gm bred at animal house of Department of Pharmacology, University of Karachi were used. The mice were given water and standard diet ad libitum for 21 days. They were kept under constant environmental conditions 23±2°C.[10] All animals were equally divided into three groups, one group served as control, second as standard (lorazepam) and third as treated with ethanolic extract of *Cleome brachycarpa*. Animals were handled as per specifications provided in Helsinki Resolution 1964 and study was approved by our Board of Advanced studies and research vides Resol. NO.10 (6) dated 26-09-2012 & 16-10-2012.

**Dosing Protocol:** The dosing of *Cleome brachycarpa* was on daily basis. *Cleome brachycarpa* dose 300mg/kg was adjusted in milligram (mg) according to weight of mice which was in grams. Stock solution of 250mg/10ml in DMSO was prepared and dose was administered by serial dilution method orally. Control mice were given similar milliliter (ml) of DMSO. Standard drug used was Lorazepam 2mg/60kg that means 0.3mg/kg, this dose was adjusted according to weight of mice in milligrams. Stock solution was prepared 12mg/60ml in DMSO and dose was administered by serial dilution method orally.

### CNS Screening Test:

**Cage Crossing Test:** Transparent, plexiglass cage (26×26×26 cm) with saw dust covered floor was used to monitor the exploratory activity of mice. The mice of all 3 groups were first individually customized with the apparatus by placing them one at a time in it for 5 minutes. After they got acquainted with setting, the numbers of cage crossings were counted for 5 minutes. [12]

**Head Dip Test:** The Head dip test or hole-board test is an exploratory behavioral test used to evaluate different anxiety related activity in rodents. The apparatus consists of an enclosed

wooden rectangular box (35cm×45cm×45cm). The holes are 2.5cm in diameter and found in all walls. [13] The mice that were ignorant of the apparatus were placed in the centre area and allowed to freely explore for 5 minutes. The number of times the mouse stuck out its snout was noted.[14]

**Forced Swim Test:** It measures the antidepressant effects of drugs. It consists of cylindrical container made of glass containing 8cm water and maintained at 22-25° C temperature. [15] The mouse is placed in a cylinder filled with water, so that feet of animal do not touch the bottom. The test is based on the assumption that the animal will try to escape from stressful stimuli, it does so by swimming actively. When animal stops swimming and floats on surface it shows a state of despair. Normally it is performed for 5 minutes in mice. The time at which immobility is achieved is recorded. [16]

**Open Field Test:** Open field test is used to assess emotional behavior in rodents. It can be used to measure exploratory activity, locomotor activity and gives idea of initial anxiety related behavior in rodents. [17] The mice were held gently by the tail and placed in centre of arena in open field. The activity of mice was observed for 10 minutes. During this experiment we observed the number of times the mice moved in the centre square using all 4 paws and the number of peripheral squares crossed by mice on all 4 paws.

**Light and Dark test:** Rodents generally favor dark areas. This test was used to assess anxiety behavior in rodents. The instinctive conflict between risk avoidance and exploratory drive is thought to inhibit exploration. The important feature that is monitored is the change in willingness to explore the lightened unprotected area. Each mouse is transferred individually in the centre of the brightly illuminated compartment. Mice are allowed to freely explore the compartments moving from light to dark. Record the transitions made by the mouse in the dark compartment. This test is conducted for 10 minutes. [18]

**Statistical Analysis:** By taking mean of all the values they are compared with means of control and standard drug and by student significance t-test the significance of difference between means are determined. A value of  $p < 0.05$  is considered significant,  $p < 0.001$  as more significant and  $p < 0.0001$  as highly significant. By Alcarz and Jimenez method all statistical procedures are performed. [18]

## RESULTS AND DISCUSSION

Herbal preparations have been used since ancient times as a source of medicinal agent because of their therapeutic efficacy, low cost and safety. *Cleome brachycarpa* belonging to family cleomaceae has not been vastly investigated although it has been shown to possess a number of therapeutic effects. Table 1.1 shows when 300mg *Cleome brachycarpa* was administered once daily, significant decrease in cage crossing activity was observed after 7, 14 and 21 days. From the above we can conclude that *Cleome brachycarpa* possess CNS depressant effects. Previous studies show that locomotor activity is controlled by peripheral signals from spinal cord and brain area which plays a role in controlling movement and posture is cerebellum. [19]

Decreased locomotor activity indicates depressed CNS activity. It is known that GABA is major inhibitory neurotransmitter in CNS. Different anxiolytic drugs mediate their action by binding to GABA<sub>A</sub> receptor by potentiating gabanergic inhibition in CNS by opening chloride channels and causing hyperpolarization which reduces firing rate of critical neurons in brain or drug directly activates GABA receptor. [20] In *Cleome brachycarpa* the CNS depressant activity may be due to trinortriterpenoid dilactone, deacetoxy brachycarpone, cabralealactone or ursolic acid. Further work needs to be done to determine the mechanism of action.

This is further verified by Open field test. The open field test is used to measure exploratory behavior, locomotor activity and anxiety in rodents. [21] The central square crossings indicate anxiety and exploratory behavior. [22] Increase frequency indicates high exploratory behavior and low anxiety. Thigmotaxis is a phenomenon in which the mouse tries to stay near proximity of wall's (peripheral crosses) due to fear factor and anxiety. [23]

Table 1.2 shows the number of central square crosses were initially decreased by *Cleome brachycarpa* but after 14 days increase was observed in central square crossing. Table 1.3 shows the numbers of peripheral square crossed were initially increased which were then decreased till day 21. Initially numbers of central square crosses were decreased which were then increased showing anxiolytic effect. Similarly for peripheral squares the number of crosses were initially increased which was then decreased showing anxiolytic activity.

Table 2.1 shows that after *Cleome brachycarpa* intake initially there was increase in head dips followed by gradual decrease on day 14 and 21. On initial exposure to apparatus, the animal tries to find an escape route due to fearful and neophobic response.[24] Stressful condition of animal is further confirmed by elevated levels of corticosteroids in adult rats following first exposure to apparatus.[25] If it is assumed that on exposure to apparatus, anxiety develops due to state of fear so decrease in number of dips shows relieve from anxiety or reduced fear.[26] This postulation supports our above results that our extracts possess anxiolytic effect.

Forced swim test is not only an indicator of anti-depressant effect of drugs but it is also used as an indicator of depression in rodents.[27] The depressive state is represented when mice become immobile after period of vigorous activity. [15] Depression is basically defined in clinical terms as pathological complex of psychological, neuroendocrine and somatic symptoms. [28]

Table 3.1 indicates increased immobility time when *Cleome brachycarpa* was administered for 21 days further confirming that it relieves anxiety. The light and dark test is done to determine the anxiolytic or anxiogenic effects of drugs. It is based on instinctive nature of mice and rats to repel brightly illuminated area as well as to determine the exploratory response of rodents when they are exposed to such stressors as light and changed environment. [29]

Research studies have shown that an animal who is stressed or in fear or has anxiety tends to stay for prolonged period in darker area. He will not prefer to move and explore about the white box and peaking between two boxes will also be low. On the other hand anxiolytic drugs increase the number of transitions and the time spend in the white area. [30] Table 2.2 showed increased numbers of transitions after acute dosing of *Cleome brachycarpa* showing its anxiolytic effect.

## CONCLUSION

From the current study the ethanolic extract of *Cleome brachycarpa* after acute dosing has been screened for neuropharmacological profile and it has been suggested that *Cleome brachycarpa* possesses significant anxiolytic property as well as CNS depressant activity. This work can be further extended to evaluate the effect of *Cleome brachycarpa* on specific regions of brain and neurotransmitters.

Table 1.1 : Effect of <i>Cleome brachycarpa</i> on exploratory activity									
Effect of <i>Cleome brachycarpa</i> on Cage crossing									
Number of cage crosses									
	Day 7			Day 14			Day 21		
Groups	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)
control	45.3± 2.31			45.2 ± 1.75			47.1 ± 1.73		
standard	27.7± 1.77			13.9± 1.66			7.8 ± 1.55		
<i>Cleome brachycarpa</i>	37.7 ± 2.06	***0.000	***0.000	36.9 ± 1.6	***0.000	***0.000	34 ± 2.26	***0.000	***0.000
			0			0			

Table 1.2: Effect of <i>Cleome brachycarpa</i> on exploratory activity									
Effect of <i>Cleome brachy carpa</i> on Central square crosses in Open Field Test									
Number of Central square crosses									
	Day 7			Day 14			Day 21		
Groups	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)
control	33.4 ± 1.17			32.1 ± 1.45			30.7 ± 1.06		
standard	17.2 ± 1.32			12.3 ± 1.37			5.00 ± 1.49		
<i>Cleome brachycarpa</i>	27.0 ± 1.49	***0.000	***0.000	52.7 ± 1.89	***0.000	***0.000	53.1 ± 1.52	***0.000	***0.000
			0			0			

Table 1.3: Effect of <i>Cleome brachycarpa</i> on exploratory activity									
Effect of <i>Cleome brachy carpa</i> on Peripheral square crosses in Open Field Test									
Number of Peripheral square crosses									
	Day 7			Day 14			Day 21		
Groups	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)
control	137 ± 1.25			131.6 ± 1.78			138.7 ± 1.49		
standard	167.1 ± 2.6			50.8 ± 2.94			15.1 ± 3.07		
<i>Cleome brachy carpa</i>	194.4 ± 2.41	***0.000	***0.000	167.1 ± 1.85	***0.000	***0.000	152.7 ± 1.77	***0.000	***0.000
			0			0			

Table 2.1 : Effect of *Cleome brachycarpa* on Anxiolytic Activity

Effect of <i>Cleome brachycarpa</i> on Head Dip Activity									
Number of Head Dips									
Groups	Day 7			Day 14			Day 21		
	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)
control	34.5 ± 1.58			33.8 ± 1.62			32.7 ± 2.0		
standard	34.4 ± 1.17			22.6 ± 1.71			12.8 ± 1.75		
<i>Cleome brachycarpa</i>	34.90 ± 1.52	IS 0.5	IS 0.4	32.3 ± 1.77	*0.064	***0.000	26.10 ± 2.5	***0.000	***0.000
						0			

Table 2.2 : Effect of *Cleome brachycarpa* on Anxiolytic Activity

Effect of <i>Cleome brachycarpa</i> on Light and Dark Activity									
Time spent in Light Box (seconds)									
Groups	Day 7			Day 14			Day 21		
	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)
control	473.2 ± 2.62			487 ± 1.49			485.1 ± 1.45		
standard	175.8 ± 3.33			124 ± 3.4			16.1 ± 3.48		
<i>Cleome brachycarpa</i>	448.5 ± 1.08	***0.000	***0.000	402.4 ± 1.9	***0.000	***0.000	386.5 ± 1.35	***0.000	***0.000
			0			0			

Table 3.1 : Effect of *Cleome brachycarpa* on Anti-Depressant Activity

Effect of <i>Cleome brachycarpa</i> on Forced Swimming Test									
struggling time (seconds)									
Groups	Day 7			Day 14			Day 21		
	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)	Mean ± SD	P( control)	P( STD)
control	92.7 ± 2.00			84.8 ± 1.03			95.8 ± 1.03		
standard	60.1 ± 1.66			30.10 ± 1.66			12.1 ± 1.52		
<i>Cleome brachycarpa</i>	83.3 ± 1.77	***0.000	***0.000	71.7 ± 1.16	***0.000	***0.000	54.5 ± 1.08	***0.000	***0.000
			0						

Values are mean ± S.D; N=10= number of animals; \*p<0.05=significant; \*\*\*p<0.0001 = highly significant; IS = insignificant difference; Following t-test and ANOVA df (2, 29)

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