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Research Article

EVALUATION OF CARALLUMA FIMBRITA FOR ANALGESIC, ANTI INFLAMMATORY AND ANXIOLYTIC ACTIVITIES

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ABSTRACT

The aim of the present study was to evaluate the Analgesic, anti inflammatory & anxiolytic activities of the Caralluma fimbriata extract. In the evaluation of analgesic activity the model used was Eddy's hot plate method in which the animals treated with *Caralluma fimbriata* and standard Pentazocin has significantly increased the latency period of jumping & paw licking when compared with control group animals. The anti – inflammatory activity was screened by Carageenan induced paw edema model in which the animals treated with testing drug and standard indomethacin has significantly reduced the inflammation when compared with carageenan induced inflammatory positive control group animals. In the evaluation of anxiolytic activity the animals treated with the testing drug and standard diazepam has significantly raised the time spent in open arm and a number of entries when compared with control group animals in elevated plus maze model. Since all the animal models used in this study were well established models and used by many authors, so we can conclude that the extract of Caralluma fimbriata has the analgesic, anti inflammatory and anxiolytic activities.

Keywords: Analgesic, Anti-inflammatory, Anxiolytic, Caralluma fimbriata and Elevated plus maze

INTRODUCTION

There have been several attempts to define pain. McCafferey (1972) states that "pain is whatever the experiencing person says it is, existing whenever he/she says it does". The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"[1]. Pain is the most common reason for physician consultation. It is a major symptom in many medical conditions. It can significantly interfere with a person's quality of life and general functioning [2]. It is part of the body's defense system, producing a reflexive retraction from the painful stimulus, and tendencies to protect the affected body part while it heals, and avoid that harmful situation in the future [3, 4]. Pain is in general seen as nociceptive, inflammatory or a neuropathic response. Pain is primarily managed with analgesics. Opioid analgesics are commonly used for treatment of pain. Although opioids are strong analgesics, there are other drugs used for the treatment of pain. Antidepressants and antiepileptics are also used in painmanagement[5].

Inflammation is the body's immediate response to damage to its tissues and cells by pathogens, noxious stimuli such as chemicals, or physical injury[6]. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation can be classified as either acute or chronic status depending on the onset time. Acute inflammation is the primary response of the body to injurious stimuli and it involves the local vascular and immune response. On

the other hand, chronic inflammation is a pathological condition characterized by progressive destruction and recovery of the injured tissue from the inflammatory response[7].

Anxiety is an unpleasant emotional experience of daily living characterized by a sense of apprehension, uneasiness or impending distress; this feeling is usually associated with changes in the autonomic nervous system and behavior and it affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade [8, 9].

Currently the most widely prescribed medications for anxiety disorders are the benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiation of other central depressant drugs and dependence liability[10]. Also prolonged use of tranquilizers and psychotropic drugs in the management of anxiety, stress and psychosomatic disorders leads to a variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects. Moreover such agents primarily relieve the symptoms and offer palliative relief of a temporary nature [11].

Usually inflammation always associated with pain and many studies reveal that anxiety & depression elevate the inflammation and in turn pain too. Many allopathic, ayurvedic drugs were available to treat inflammation, pain & anxiety separately but the multiple drug therapy causes many side effects. All these factors provoked me to search for a drug to treat all the three symptoms. Based on the traditional system of usage of medicine a few drugs are available to manage inflammation, pain & anxiety. Among them *Caralluma fimbriata* is one of the drug to manage all the three targets. So we selected this *Caralluma fimbriata* for evaluation of Analgesic, Anti inflammatory & Anti anxiety activity.

MATERIALS & METHODS

Drugs and chemicals

Reference standards such as diazepam tablets, pentazocine injections and indomethacin capsules procured from Ranbaxy laboratories. All Other chemicals used for this investigation were of analytical grade from S.D Fine chemicals, Mumbai, India.

Animals

Albino Wistar rats weighing 150 ± 25 g of either sex were used for the study in different models. The animals were procured from the National institute of Nutrition (Hyderabad) at least 2 weeks prior to the study, so that animals could acclimatize to the new environment. Animals kept in well-maintained room under standard hygienic conditions. Commercial pellet diet and water were made available *ad libitum*. They were housed in propylene cages (32 x 24 x 16 cm) with stainless steel grill top, bedded with rice husk.

Preparation of Extract

The leaves of *Caralluma fimbriata* were dried under shade at room temperature for 3 days and powdered and the powder was used for preparation of methanolic extract. A 95% w/v methanolic extract was prepared by the Soxhlet extraction method. The dried powder was extracted with 95% methanol for 12 h using a Soxhlet apparatus. The combined extracts were concentrated at 40^0 C to obtain light brown residue. The yield obtained from the above process was found to be 11%. The extract was preserved in a refrigerator.

Selection of Doses and Preparation of Drug for Study

Since the lethal dose was found at 2000mg/kg body weight, the $1/10^{th}$ of the preceding dose i.e. 100mg/kg body weight was taken as the test dose for this study and the doubling of the dose i.e. 200mg/kg body weight also tested to find out was there any dose dependent pharmacological effect or not.

Screenings of anti-inflammatory activity

Carageenan induced hind paw edema in rats[12, 13]

Albino Wistar rats weighing between 150-200gms were divided into 5 groups of 6 rats each; three animals being housed in a labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum

Grouping:

Group I: Animals were administered 0.1ml saline p.o

Group II: Animals were administered 0.1ml saline p.o

Group III: Animals were administered standard (Indomethacin 10 mg/kg) p.o

Group IV: Animals were administered *Caralluma fimbriata* (100 mg/kg) p.o

Group V: Animals were administered *Caralluma fimbriata* (200 mg/kg dose) p.o

Procedure: All rats of II, III, IV & V (except I group) groups were injected with 0.1ml of Carageenan (1%) in normal saline into the sub planter area of right hind paw. All the drugs were given orally 1hr prior to Carageenan injection.
Paw volume was measured by the mercury plethysmograph at 0, 1, 2, 3, 6 h after the Carageenan injection.

Screening of analgesic activity [14, 15]
Eddy's hot plate method
Grouping: Albino Wistar rats weighing between 150-200gms were divided into 4 groups of 6 rats each; three animals being housed in a labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum.

Group I: Animals were administered 0.1ml saline p.o
Group II: Animals were administered standard reference Pentazocin (10 mg/kg) i.p.
Group III: Animals were administered *Caralluma fimbriata* (100 mg/kg) p.o
Group IV: Animals were administered *Caralluma fimbriata* (200 mg/kg) p.o

Procedure: In this model prior to the experiment the hot plate was set for a temperature 55^0C and the animals were treated with respective drugs 30 mins., prior to the recording the response. The time for licking paws or jumping in hot plate was recorded as a response, prior and 0, 30, 60, 90 120 min after administration of the respective drugs.

Screening of Anti Anxiety activity
Grouping: Albino Wistar rats weighing between 150-200gms were divided into 4 groups of 6 rats each; three animals being housed in a labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum.

Group I: Animals were administered 0.1ml saline p.o
Group II: Animals were administered standard reference Diazepam (2 mg/kg, p.o.)
Group III: Animals were administered *Caralluma fimbriata* (100 mg/kg) p.o
Group IV: Animals were administered *Caralluma fimbriata* (200 mg/kg) p.o

Elevated Plus-Maze Model[16, 17, 18]
The plus-maze apparatus consisting of two open arms (30 x5 x 0.2cm) and two closed arms (30cm x 5cm x 15cm) extending from a central platform and were elevated to a height of 45cm above the floor. The entire maze was made up of clear plexi glass. Prior to the test, animals were treated with respective drugs. One hour after the treatments, each rat was individually placed on the center of the elevated plus maze with its head facing the open arm. During the entire experiment, rats were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus-maze could invoke maze anxiety. During the 5 min experiment, following behavior of the mouse was recorded;
- Number of entries into the open arm
- Number of entries into the closed arm
- Time spent in the open arm and
- Time spent in the closed arm

Every time before placing each animal, the arena was washed with 5% alcohol to eliminate the possible bias due the odor left by the previous animal.

STATISTICAL ANALYSIS
All the data's were analyzed using One-Way ANOVA method followed by Dunnet's / Tukey's test. All values were reported as mean ± SEM. $P \leq 0.05$ was considered to be statistically significant**.**

RESULTS

Anti – inflammatory activity
Carageenan induced paw edema in rats:
In carageenan induced paw edema *Caralluma fimbriata* significantly inhibited the edema in a dose dependent manner as shown in Table.1. The paw volume in normal control group rats on 2^{nd} hr was found to be 0.2148 ± 0.0122 ml. The paw volume in rats pretreated with lower dose of *Caralluma fimbriata* (100 mg/kg/day), higher dose of *Caralluma fimbriata* (200 mg/kg/day) and indomethacin (10 mg/kg/day) at 2^{nd} hr were found to be 0.191 ± 0.0061 ml, 0.158 ± 0.0042** ml and 0.1369 ± 0.0054** ml

Analgesic activity
Eddy's hot plate: *Caralluma fimbriata* showed maximum analgesic activity at 60, 90 min for 100 and 200mg/kg dose. The reaction time in normal control group at 60, 90 min were found to be 3.52±0.002, 4.08±0.161 Sec. The reaction time (paw licking / jumping response) in rats pretreated with a lower dose of *Caralluma fimbriata* (100mg/kg), higher dose of *Caralluma fimbriata* (200mg/kg/day) and Pentazocine (10 mg/kg) at 60, 90 min were found to be 9.26±0.851, 7.16 ± 0.193, 9.82 ± 0.894 and 8.60 ± 0.992, 9.12 ±0. 372, 14.12±3.182 respectively, when compared to control group rats. The duration of

analgesic effect was more in 200 mg/kg compared to 100 mg/kg and reference drug pentazocine at 10 mg/kg dose significantly increased the reaction time at 90 minutes as shown in Table 2.

ANTI ANXIETY ACTIVITY

Elevated Plus-Maze Test: The classic anxiolytic benzodiazepine; diazepam has long been reported for its anxiolytic activity in rat with EPM. In our study also, a significant anxiolytic effect was recorded with diazepam as it increased the number of entries in open arms and the time spent in open arms along with a significant decrease in time spent in closed arms. *Caralluma fimbriata* at doses of 100 mg/kg, and 200 mg/kg showed significant anxiolytic activity by increasing the number of entries in open arms along with time spent in open arms and significant reduction in time spent in closed arms. The effect of *Caralluma fimbriata* (100, 200 mg/kg) on the number of entries in closed arms was insignificant. The anxiolytic activity shown at higher doses of *Caralluma fimbriata* (200 mg/kg) was comparable with Diazepam 2 mg/kg. p.o.

Table 1: Anti-inflammatory effect of *Caralluma fimbriata* on carageenan induced paw edema in rats

Treatment	Paw volume in ml at different Hrs (Mean ± S.E.M.)				
	0	1	2	3	6
Normal Control	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058	0.101± 0.0058
Inflammatory control	0.1225 ± 0.0079^{+++}	0.1876 ± 0.007^{+++}	0.2148 ± 0.0122^{+++}	0.2083 ± 0.0094^{+++}	0.165 ± 0.0076^{+++}
Indomethacin 10mg/kg, p.o.	0.1249 ± 0.0061	0.1427 ± 0.0071**	0.1369 ± 0.0054**	0.1442 ± 0.007**	0.1449 ± 0.0060
Caralluma fimbriata (100mg/kg)	0.1210 ± 0.0186	0.152 ± 0.008	0.191 ± 0.0061	0.196 ± 0.006	0.159 ± 0.009*
Caralluma fimbriata (200mg/kg)	0.1016 ± 0.0070	0.132± 0.0057**	0.158 ± 0.0042**	0.1542 ± 0.0071**	0.1542 ± 0.0136

Values are expressed as (Mean±S.E.M) n=6; One way ANOVA followed by Dunnet's test.
+++ P<0.001 Vs Normal control & * P< 0.01 , ** P< 0.01 Vs Inflammatory Control

Table 2: Effect of Caralluma *fimbriata* on reaction time (sec) in Eddy's hot plate

Treatment	**Reaction time in seconds**				
	0	**30**	**60**	**90**	**120**
Control	3.51 ±0.277	3.80 ±0.343	3.52 ±0.455	4.08 ±0.161	3.93± 0.067
Pentazocine (10mg/kg)	4.11 ±0.238	6.64 ±0.430**	9.82 ±0.894**	14.12 ±3.182**	9.41± 0.650**
Caralluma fimbriata (100mg/kg)	4.02 ±0.194	5.01 ±0.332	9.26 ±0.851**	8.60 ±0.992	6.30± 0.259**
Caralluma fimbriata (200mg/kg)	3.81 ± 0.230	7.09 ± 0.523**	7.16 ± 0.193**	9.12 ± 0.372	8.21± 0.671**

Values are expressed as (Mean±S.E.M) n=6; One way ANOVA followed by Dunnet's test.
** P< 0.001 Vs control, * P< 0.05 Vs control.

TABLE 3: Effect of *Caralluma fimbriata* on Elevated Plus-Maze Model

TREATMENTS	Elevated plus maze model			
	NUMBER OF ENTRIES (COUNTS/5MIN)		TIME SPENT IN (SEC/5MIN)	
	OPEN ARM	CLOSED ARM	OPEN ARM	CLOSED ARM
Control	3.66±0.55	13.16±1.22	29.16±7.94	218.16±14.28
Diazepam (2 mg/kg)	12.16±1.04***	15.16±1.04	137.16±9.33***	142.83±7.71***
Caralluma fimbriata (100 mg/kg)	4.16±1.0	12.17±1.90	37.17±11.81	216.33±15.71
Caralluma fimbriata (200 mg/kg)	7.66±0.84*	12.0±0.93	89.83±12.13**	170.66±8.55*

Values are expressed as Mean±S.E.M from 6 rats. *P*<0.05 *, <0.01 ** and <0.001 * as compared to control group**

DISCUSSION

The development of edema in the paw of the rat after injection of carageenan is a biphasic event. The initial phase of the edema has been attributed to the release of histamine and serotonin, the edema maintained during the plateau phase to kinin like substances and the second accelerating phase of swelling to the release of prostaglandin like substances. Inhibition of edema observed in various inflammatory models induced experimentally in the present study may, therefore be attributed to the ability of the *Caralluma fimbriata* to inhibit various chemical mediators of inflammation like histamine and 5-HT during the initial phase[13].

In the present study *Caralluma fimbriata* significantly increased the reaction time in the hot-plate test suggesting its central analgesic activity; the probable mechanism could be by inhibition of prostaglandin synthesis. Prostaglandins play a significant role in different phases of inflammatory reactions and elicit pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other pain provoking stimuli.

The elevated plus-maze was one of the most widely used models of animal anxiety, having been employed by many research laboratories in the past 6 years and has been extensively validated for use with rats and mice[19, 20]. The test is principally based on the observations of Montgomery showing that exposure of animals to an elevated maze alley evokes an approach-avoidance conflict that is considerably stronger than that evoked by exposure to an open maze allay. Elevation of the maze causes great fear and avoiding conflict. EPM consisting of two (opposite) open and two walled allays. The animal will explore the different allays (total number of entries). The open arms are more averse than the closed ones, as revealed by a preference of the animals to explore the closed allays. Anxiolytic drugs will help to overcome the fear induced inhibition of open allay exploration. Diazepam a standard anxiolytic used clinically and is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic-like effects, even when the compound being screened does not act via benzodiazepine receptors. As expected standard diazepam significantly increased the number entries and time spent in open arm. *Caralluma fimbriata* at doses of 100 mg/kg & 200 mg/kg significantly increased the time spent and arm entries in open arms and decreased the time spent in closed arms compared to control. The time spent in the neutral zone is also reduced by both the doses compared to control group. Decreased aversion to open arms compared to control group indicates the anxiolytic activity of stem bark of *Caralluma fimbriata and* the magnitude of the anxiolytic effect of 200 mg/kg and

100 mg/kg extracts of *Caralluma fimbriata* were comparable to that of standard drug diazepam 2 mg/kg p.o.

CONCLUSION

The findings in this study suggest that the *Caralluma fimbriata* possess Anti- anxiety, anti- inflammatory and analgesic activity. The results have been obtained in carefully controlled experiments with laboratory animals where psychological factors can presumably be ruled out. In all the tests the responses have been assessed by actual measurement and not by subjective comparisons which may be influenced by the observer. Therefore the statistical validity of the findings has been proven and they provide a scientific foundation for the use of the biologically active ingredients of *Caralluma fimbriata* in anxiety, inflammatory and pain conditions and explain the clinical effectiveness of the *Caralluma fimbriata*.

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