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Antianxiety activity of *Carissa congesta* weight leaves

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ABSTRACT

The aim of the present study was to investigate the antianxiety activity of various extracts of *Carissa congesta* leaves. In the present investigation, leaves of the plant were extracted using solvents in order of increasing polarity viz., petroleum ether (60-80?C), chloroform,ethyl acetate and ethanol. All the crude extracts were evaluated for anti-anxiety activity in mice using elevated plus maze apparatus. Among all these extracts, ethyl acetate and ethanolic extract exhibited significant anti-anxiety activity at a dose of 400 mg/kg in mice with respect to control as well as standard (diazepam, 4 mg/kg).It was observed that at the dose of 400 mg/kg dose the Ethyl acetate and Ethanolic extract shows increase the time spent and the number of arm entries in the open arms of the elevated plus-maze. As phytochemical screening of ethanolic extract showed presence of polyphenols, i.e., flavonoids and tannins, thus, these constituents might be responsible for anxiolytic potential of *Carissa congesta*.

KEYWORDS: *Carissa congesta*, antianxiety, diazepam, elevated plus maze.

1. INTRODUCTION

Anxiety is the displeasing feeling of fear and concern. When anxiety becomes excessive, it may be considered as an anxiety disorder[1]. Anxiety disorders are the most common emotional disorders affecting people worldwide. Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or real threat. Anxiety is a wide spread, affects one eighth of the total population worldwide, with the life time prevalence ranging from 13.8 to 28.87 % in western countries. Individuals aged between 10 to 25 years are at high risk for developing an anxiety[2].

The existing anxiolytic drugs such as benzodiazepines like diazepam, nitrazepam, alprazolam etc are more frequently prescribed synthetic drugs for the treatment of anxiety, depression, epilepsy and insomnia. These medications cannot fully cure anxiety disorder they can, to a great degree relives the symptoms and reduces their occurrence[3]. There are limitations to use synthetic anxiolytic drugs due to their unwanted side effects such as sedation, addiction and deterioration of cognitive functions. Therefore the development of new pharmacological agent from plant sources in the treatment of anxiety is of great interest.

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Carissa congesta weight commonly known as karvanda is rank-growing, straggly, woody, climbing shrub, usually growing to 10 or 15 ft (3-5 m) high, sometimes ascending to the tops of tall trees; and rich in white, gummy latex. The branches, numerous and spreading, forming dense masses, are set with sharp thorns, simple or forked, up to 2 in (5 cm) long, in pairs in the axils of the leaves. Traditionally the plant is used as astringent, appetizer, antipyretic; lessen-thirst, biliousness and in diseases of the brain[4,5]. Earlier studies have shown that various parts of *Carissa congesta* possess various activities like cardiotonic[6], anticonvulsant[7], histamine releasing[8], neuropharmacological and diuretics[9],antipyretic[10] ,anticancer[11],hepatoprotective[12] etc.

The aim of present study was to evaluate the Antianxiety activity of various extract of leaves of *Carissa congesta* in experimental animal models with a view to provide a pharmacological justification for the folkware use of the plants leaves in the management of brain diseases.

2. MATERIAL AND METHODS

2.1. Plant material:

The leaves of *Carissa congesta was* collected from the Parner, Maharashtra, India. The leaves were identified by Dr. S.Jayanthi, Joint Director, Botanical Survey of India; Pune with a voucher specimen **(CCA01)** has been kept in herbarium botanical survey of India, Pune.

2.2. Preparation of extracts:

The leaves of *Carissa congesta* were collected and air dried under shade and then coarsely powdered with the help of mechanical grinder. 500gms of powdered materials were evenly packed in the soxhlet apparatus. It was then extracted with various solvents from nonpolar to polar such as petroleum ether, ethyl acetate, chloroform, ethanol and aqueous successively.

2.3. Preliminary phytochemical evaluation:

The preliminary phytochemical screening of petroleum ether, ethyl acetate and ethanolic extracts of *Carissa congesta* leaves were carried out for qualitative identification of type of phytoconstituents present[13].

2.4. Acute toxicity studies

Swiss albino mice of either sex (18-22 g weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD and animals were observed for mortality and behavioral changes.

2.5. Drugs and chemicals

Diazepam (Ranbaxy Laboratory limited.), Petroleum ether, chloroform, ethyl acetate Ethanol (Loba Chemicals Mumbai.).

2.6. Antianxiety activity

The petroleum ether, ethyl acetate and ethanolic extracts of *Carissa congesta* leaves were tested for antianxiety activity using elevated plus maze.

2.7. Treatment

Animals were divided into five (I-V) groups. Group I was a control and was given vehicle, carboxy methyl cellulose (2%), Group II was given standard drug, diazepam (4 mg/kg,) Group III-V were treated as test groups and were given petroleum ether (60-80°C), ethyl acetate and ethanol extracts of leaves of Carissa congesta at dose100mg/kg,200 mg/kg and 400mg/kg respectively. All the test solutions, standard drug and control were administered orally 45 minutes prior to elevated plus maze test[14].

2.8. Antianxiety Activity

2.8.1. Elevated plus maze (EPM) model

The apparatus comprises of two open arms (35x5cm) and two closed arms (30x5x15cm) that extend from a common central platform (5x5cm). The floor and walls of the closed arms are made of wood and painted black. The entire maze is elevated to a height of 50 cm above the ground level. Rats weighing (150 – 200gms) were housed in a pair of 10 days prior to the test in the apparatus. During this time the rats were handled by the investigator on alternate days to reduce stress. 30 min and 60min after oral administration of the drug treatment, each rat was placed in the center of the maze facing one of the enclosed arms. During five minutes session, number of entries into open arm and time spent in the open arm were noted,[15]. The procedure was conducted preferably in a sound attenuated environment.

2.9. Statistical analysis

All the data were given as means +_S.E.M. Data were analysed by one way ANOVA .Whenever ANOVA was significant, further comparisons between vehicle and drug treatment groups were performed using Dunnette's multiple comparison test. The level of statistical significance adopted was $P < 0.05$.

3. RESULTS

Table 1 shows results of phytochemical screening of various extracts of *Carissa congesta* leaves. The behavioural alterations induced by the *Carissa congesta* leaves extract in the EPM provided anxiolytic effect because the ethyl acetate and ethanolic extract of *C.congesta* leaves at a dose of 400 mg/kg significantly increased the arm entries in open arms and decreased the time spent and arm entries in closed arm.

Table : 1. Phytochemical analysis of *Carissa Congesta* leaf extract

Sr.No.	Phytochemical Constituents	Pet.ether extract	Ethyl acetate extract	Ethanol extract
1	Steroids	+	+	+
2	Saponins	+	+	+
3	Tannins	+	-	+
4	Alkoloids	+	-	+
5	carbohydrates	-	+	+
6	Proteins	+	+	-
7	Amino acids	-	-	+
8	Flavonoids	-	+	+
9	Diterpenes	-	-	+
10	Phenols	-	+	+

Table : 2. Antianxiety activity of *Carissa congesta* leaves

Sr. No.	Groups	Treatment	No. of Entries in Open arm	Avg. time spent in open arm in Sec
1	Control	D/W10ml/kg, p.o.	4.50±0.42	8.00±0.36
2	Standard	Diazepam. 4 mg/kg ,i.p	8.33±0.55**	14.83±0.47**
3	PECC	100 mg/kg, p.o.	5.00±0.36ns	7.83±0.30ns
		200 mg/kg, p.o.	6.00±0.36*	9.50±0.42*
		400 mg/kg, p.o.	6.16±0.16**	9.66±0.33*
4	EECC	100 mg/kg, p.o.	4.33±0.33ns	8.00±0.25ns
		200 mg/kg, p.o.	6.33±0.21**	9.66±0.33*
		400 mg/kg, p.o.	6.66±0.33**	9.83±0.30**
5	EECC	100 mg/kg, p.o.	4.83±0.30ns	8.16±0.30ns
		200 mg/kg, p.o.	6.50±0.22**	9.66±0.42*
		400 mg/kg, p.o.	6.83±0.30**	10.00±0.36**

** P< 0.05, **P<0.01 Values are Mean± SEM , n=6,when compared with control by using one way ANOVA followed by Dunnette's multiple comparison test*

4. DISCUSSION

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms[16]. The ethyl acetate and ethanoicl extract of Carissa congesta (400 mg/kg), markedly increased the percentage of average time spent by the animals in the open arms.

The anxiolytic effects of ethyl acetate and ethanolic extract of Carissa congesta may be related to their flavonoid content. Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine such as *Passiflora coerulea* [17] this effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors[18]. Furthermore a sedative effect on the central nervous system has been shown for quercetin and isoquercetin glycosides in mice[19,20].

However, further studies are required to identify the phytoconstituent responsible for the observed anxiolytic effect of ethyl acetateand ethanolic extract at dose 400mg/kg and to explain anxiolytic mechanism.

5. CONCLUSION

These experimental results have established a pharmacological evidence for the folklore claim about the usefulness of *C.congesta* leaves extract. Further the study of isolation of active principles responsible for such activity is essential.

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