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Minor Tranquilizing Activity of Plant Chrozophora Plicata

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Abstract: The study was designed to investigate the minor tranquilizing (anxiolytic) effects of ethanolic extract of chrozophora plicata leaves (600mg/kg) against experimentally induced anxiety models such as elevated plus maze, and open field test in albino rats. In the elevated plus maze, three groups of albino rats, each group containing three animals are used. The control group, test group (chrozophora plicata 600mg/kg, p.o) and standard (alprazolam 0.5mg/kg) treated animals are placed in the center of elevated plus maze apparatus one by one head facing towards open arm and the preference of animals towards open and closed arms are noted for a period of 5minutes. In open field test, the rats are placed in the center of open field test apparatus consisting of a block made up of thermocole and the behavioral responses of anxiety such as ambulation, rearing, self grooming and activity in the center were noted for a period of 5minutes with control, test extract and alprazolam treated rats. The crude ethanolic extract of chrozophora plicata (600mg/kg) showed significant anxiolytic activity in albino rats by increasing in time spent by the animals in open arms (76.66±1.58seconds) of elevated plus maze apparatus in comparision with the time spent in open arms with control animals (24.6± 0.62seconds). The behavioral responses of anxiety in open field test such as ambulation, activity in center and self grooming shown by rats with text extract chrozophora plicata (600mg/kg) was found to be less (2.99) when compared to control group rats (12.625). Hence it indicates the given test ethanolic extract of chrozophora plicata leaf (600mg/kg) possesses anxiolytic activity. Phytochemical screening of chrozophora plicata leaf extract indicates the presence of Flavonoids, alkaloids, tannins and glycosides. Therefore the antianxiety activity of crude chloroform extract of chrozophora plicata may be due to tannins and flavonoids.[1]
Key words: Anxiolytic activity, Ethanolic extract, Chrozophora plicata.

Introduction

Anxiety is not only a normal response, it's a necessary response.[2] Normal anxiety is an emotion that helps organisms defend against a wide variety of threats. There is a general capacity for normal defensive arousal, and subtypes of normal anxiety protect against particular kinds of threats. These normal subtypes correspond somewhat to mild forms of various anxiety disorders.[3] Anxiety disorders are characterised by distorted beliefs about the dangerousness of certain situations and/or internal stimuli.[4] The phytochemical profile of this plant chrozophora plicata reveals the presence of sterols, alcohols, hydrocarbons. flavonoids, lignans, coumarins, tannins, phenanthrenes, quinones, phenolic acids, alkaloids, cyanogenic glucosides and glucosinolates. It is evident from the literature that flavonoids and tannins posseses antianxiety properties[5]. Since there are no reports of isolation of active anxiolytic principles of Chrozophora plicata, an herbal ingredient present in unani medicine safi, the present study is planned to exploit the antianxiety activity of herbal plant named Chrozophora plicata, Family: Euphorbiaceae by using elevated plus maze and open field test animal models in rats.

Materials and Methods

For the present study, the leaves of chrozophora plicata were collected from the surrounding gardens of the gajwel (mandal), medak dist, Andhra Pradesh, india. After the fresh leaves were authenticated by botanist, leaf specimens have been deposited at the museum of the college. Fresh mature leaves were shade dried at room temperature, coarse powdered and extracted with ethanol by soxhlet's extraction method. Thereafter, the extracts were concentrated using electric water bath to obtain semisolids crude extract. The percentage yields of the leaf extract were found to be 10.7%. The extract was stored in airtight container in refrigerator below 10^{o}c. Appropriate concentration of stock solution of extract were prepared using distilled water and used for the following studies.

Preliminary phytochemical screening[6]

Preliminary phytochemical tests were performed for the chloroform extract of Chrozophora plicata to detect the presence of phytochemicals by following the standard methods described in the practical pharmacognosy of kokate and khandelwal. The results have been tabulated in table I.

Experimental animals

Albino rats (180-225g) were used in the experiments. They were procured from sainath agencies, musheerabad. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 10 days. Animals were housed in polypropylene cages and maintained under standard environmental conditions such as temperature (26 ± 2ºc), relative humidity (45-55%) and 12hr dark/light cycle. The animals were fed with rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum.* The study protocol was approved from the institutional animal ethics committee (IAEC) before commencement of experiment (1230/a/08/CPCSEA).

Determination of acute toxicity

The Chrozophora Plicata ethanolic extract was studied for acute toxicity study at a dose of 5 mg/kg, 50mg/kg, 300 mg/kg, and 2000 mg/kg p.o in albino mice. The extract was found safe to all the animals 12 rats. The mice are subjected to a dose of 5000 mg/kg. Even at 5000mg/kg no mortality is seen, but few symptoms of CNS depression such as sedation, drowsiness and motor incoordination is seen in all the mice at 5000mg/kg. Hence a dose of 3000mg/kg is selected as safer dose and $1/5^{th}$ of 3000mg/kg i.e. 600mg/kg is selected for our study.

Effect of Ethanolic extract of Chrozophora Plicata leaves on Elevated plus Maze induced anxiety in rats

The experiment was performed on albino rats (150-250gms) of either sex procured from sainath agencies, musheerabad.The animals were housed in colony cages at an ambient temperature of $26+2^{0}$C and, relative humidity (45-55%),with a 12h/12h light dark cycle and access to food and water ad libitum. Food was restricted during experiments.Stock solutions of alprazolam (0.5mg/kg).and ethanolic extract of Chrozophora plicata leaaves (600mg/kg) were prepared in 2% acacia suspension. Weigh and mark the animals. Divide the animals in to three groups control(C), test (T), and standard(S) each consisting of three rats. All the animals were subjected to elevated plus maze assessment of anxiety levels. The plus maze is composed of 2 open arms (16x5cms) and 2 closed arms (16x5x12cms) with an open roof and is elevated to a height of 25cms. The control group of each animal was placed individually in the centre of the maze, head facing towards the open arm and the following parameters were recorded for a period of 5 minutes.

i) Time spent in open arm.
ii) Time spent in closed arm.
iii) Total number of entries in open arm and closed arm during 5min session.

Repeat the same procedure for test and standard group rats, after 1hr of administration of ethanolic extract of chrozophora plicata (600mg/kg) and Alprazolam(0.5mg/kg) and the above parameters were recorded.

Efect of Ethanolic extract of Chrozophora Plicata leaves on Open Field Test induced anxiety in rats

Weigh and mark the animals. Divide the animals in to three groups control(C), test (T), and standard(S) each consisting of three animals. An open field apparatus, suitable for mice were made up of a thermocole (40cmsX40cms). The entire themocole block was wrapped with black sheet and the thermocole floor area was divided in to 16 squares by 6mm white lines. The apparatus was placed at a distance of 50cm from the ground. The entire apparatus was illuminated by a 40w bulb kept at distance 30cm from the apparatus and the surrounding area was kept dark. The control group of each animal was placed at the centre of the field and was left for 2min for acclimatization with the apparatus. Thereafter for the next 5min the following parameters were noted a) Ambulation (number of squares crossed by the rat)

b) Rearing (number of times the animals stands on a rear paws)
c) Activity in the centre (number of central squares crossed by the animals)
d) Self grooming (number of times the animal scratches/licks the face/paw region)
e) Fecal droppings

Repeat the same procedure for test and standard groups after 1hr of administration of ethanolic extract of chrozophora plicata leaves (600mg/kg) and Alprazolam(0.5mg/kg) and the above parameters were recorded for a period of 5 minutes.

Statistical Analysis

The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using One way ANOVA, Followed by Dunnett's test where $P<0.001$, $P<0.01$ and $P<0.05$ was considered statistically significant.

Results and Discussion

Preliminary Phytochemical screening of ethanolic extract of chrozophora plicata leaves reveals the presence of flavonoids, tannins, alkaloids and glycosides. The results are shown in table I. In elevated plus maze anxiolytic model it is observed that there is an increase in number of entries in open arm (4.66) and time spent in open arm (76.66±1.58) with chrozophora plicata treated rats in comparision with control rats (3.66 & 24.6± 0.62). The results are shown in tables II, III & IV which is a clear indication of antianxiety activity of ethanolic extract of chrozophora

plicata leaves. The Results obtained from open field test anxiolytic model is shown in tables V, VI & VII which clearly shows the reduction in behavioral responses of anxiety in chrozophora plicata treated rats with mean average value of 2.99. Whereas the mean average value of behavioral responses with control rats is 12.625 indicating the presence of anxiolytic principles in chrozophora plicata leaves. Chrozophora plicata leaves are available abundantly throughout Andhra Pradesh. The exact mechanism behind anxiolytic activity of chrozophora plicata is not understood. But it could be concluded that chrozophora plicata leaves (600mg/kg) posses anxiolytic (minor tranquilizing) activity by enhancing GABA transmission in the brain.

Table I: Preliminary Phytochemical screening of ethanolic extract of chrozophora plicata leaves.

Phytoconstituents	Ethanolic leaf extract
Carbohydrates	-
Steroids	-
Glycosides	++
Flavonoids	+++
Alkaloids	++
Tannins	+++

- **Absent**
++ **Present**
+ ++ **Present with more clarity**

Tables II, III & IV representing anxiolytic activity by using elevated plus maze apparatus.

Table II: Control: (Distilled water p.o)

S.No	Body weight(g)	No. of enteries in Closed arm	No.of enteries in open arm	Time spent in closed arm(sec)	Time spent in open arm(sec)
1	185	1	4	13± 1.32	51±0.36
2	161	1	2	11±0.14	10±1.12
3	230	2	5	15± 0.55	13 ±1.15
	Average	**1.33**	**3.66**	**13±1.05**	**24.6± 0.62**

Table III: Test (Chrozophora plicata 600mg/kg)

S.No	Body weight(g)	No. of enteries in closed arm	No .of enteries in open arm	Time spent in closed arm(sec)	Time spent in open arm(sec)
1	200	0	7	10±1.02	119±0.76**
2	185	1	6	8±0.35	87 ±1.88***
3	225	0	1	17 ±0.02	24±1.51
	AVERAGE	**0.33**	**4.66**	**8.33 ±0.74**	**76.66±1.58*** **

Table III: Standard Group :(Alprazolam 0.5 mg/Kg)

S.No	Body weight(g)	No. of enteries in closed arm	No. of enteries in open arm	Time spent in closed arm(sec)	Time spent in open arm(sec)
1	191	0	15	0±0.01**	40±1.66
2	168	1	1	8±1.89	123±1.42***
3	194	1	6	8±1.24*	115±0.55***
	AVERAGE	**0.66**	**7.33**	**5.33 ± 1.25***	**92.6±1.62*** **

P<0.001*, P<0.01** and P<0.05*** was considered statistically significant

Tables V, VI & VII represent anxiolytic activity by using open field method in albino rats. Control: (distilled water, p.o)

S.no	Body Weight(g)	Ambulation	Activity in the center	Rearing	Self grooming
1	167	7	5	0	21
2	195	6	8	0	39
3	172	16	12	3	35
	average	**9.6**	**8.3**	**1**	**31.6**

Mean average = 12.625

Test Group :(Chrozophora plicata 600mg/kg leaf extract)

S.no	Body Weight(g)	Ambulation	Activity in the center	Rearing	Self grooming
1	224	3	5	0	0
2	190	2	1	0	0
3	190	3	7	0	15
	average	**2.66**	**4.33**	**0**	**5**

Mean average = 2.99

Standard Group : (Alprazolam 0.5mg/kg)

S.no	Body Weight(g)	Ambulation	Activity in the center	Rearing	Self grooming
1	173	8	3	0	21
2	160	8	7	2	28
3	163	4	2	0	31
	average	**6.66**	**4**	**0.66**	**26.66**

Mean average = 9.49

Fig I: Elevated plus maze and open field test anxiety models in rats.

Conclusion

The data obtained from the present study indicates that the ethanolic extract of Chrozophora plicata leaves at 600mg/kg possessed significant anxiolytic effect and thus supports the use of Chrozophora plicata leaves in treatment of generalized anxiety disorder. Elevated plus maze and open field test in rats causes anxiety by increasing depolarization at the neuronal endings in rats brain. However ethanolic extract of Chrozophora plicata leaf extract had significantly reduced fear in rats by increasing in the time spent in open arm and by reducing the behavioural responses in rats. Hence it may be assumed that chrozophora plicata leaves may cause inhibition of depolarization at neuronal endings in the CNS during anxiety.

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