ANTIANXIETY ACTIVITY OF ANXY, A POLYHERBAL PREPARATION

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Summary

The Objective of present study is to evaluate the anxiolytic activity of Anxy, a polyherbal formulation, in rats. The standard anxiolytic, diazepam (0.5 mg and 1 mg/kg) and the test drug Anxy powder (5, 10 and 20 mg/kg) were suspended in 1% gum acacia solution and administered orally. In acute study the vehicle and the drugs were given sixty minutes prior to the experiment, while in the chronic study they were given twice daily for 10 days with the last dose one hour prior to the experiments (elevated plus maze and light and dark box). Acute (10 and 20 mg/kg) as well as chronic administration (5, 10 and 20 mg/kg) of Anxy increased the number of entries, the time spent, and the rears in open arms of elevated plus maze model. Similarly, in light/dark box paradigm, at higher doses the test drug increased the time spent (10 and 20 mg/kg) and the number of rears (20 mg/kg) and decreased the duration of immobility (20 mg/kg). On the other hand, chronic administration of all the doses (5, 10 and 20 mg/kg) of the test compound increased the time spent and the number of rears in bright chamber and decreased the duration of immobility. At lower doses (5 and 10 mg/kg) the test compound increased the number of entries into bright chamber. Locomotor activity in the open field test was not affected at all by the doses tested in acute study. However on repeated administration, the test drug increased the locomotor activity. These changes are similar to those induced by the standard anxiolytic diazepam. To conclude Anxy exhibited anxiolytic activity comparable to that of diazepam.

Key words: Anxiolytic, *Withania somnifera*, *Camellia sinensis, Sphaerunthus indicus, Ocimum sanctum.*

Introduction

Anxiety is a normal emotional behaviour. When it is severe or chronic, it becomes pathological and can precipitate or aggravate cardiovascular and psychiatric disorders. Although many drugs are available in allopathic medicine to treat anxiety disorders, they produce various systemic side effects or exhibit tolerance upon chronic use. In ayurvedic medicine, many plant formulations have been claimed to be free from side effects and less toxic than synthetic drugs (1).

Anxy, a polyherbal formulation containing extracts of *Withania somnifera, Camellia sinensis, Spaeranthus indicus* and *Ocimum sanctum* provided by Srinivas Natural remedies Mangalore, reportedly has antianxiety and antidepressant properties. However experimental and clinical evidence are lacking. Therefore, this study was undertaken to evaluate the effects of this polyherbal formulation on anxiety-like behaviour in rats.Three pharmacologically validated experimental models, elevated plus maze (2), light and dark box (3), and open field apparatus (4) were employed.

Materials and Methods

Animals

Male, Wistar albino rats weighing 150 to 180 g (90 to 110 days old) procured from Indian Institute of Sciences were used for this study. They are maintained under standard conditions (temperature 22 ± 2^{o}C, relative humidity 60±5% and 12 h light/dark cycle).The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. Experiments were conducted between 9:00 to14:00 h. Each rat was used only once. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Drugs

The standard anxiolytic drug, diazepam and the test drug, a dry powder of Anxy, (containing alcoholic extracts of *Ocimum sanctum, Camellia sinensis* and aqueous extracts of *Withania somnifera, Spaeranthus indicus,* provided by Srinivas Natural Remedies Mangalore, India were suspended in 1% gum acacia solution. Each drug solution was prepared freshly just before the administration. Drugs and vehicles were administered orally. The doses of each drug were selected on the basis of preliminary effects on muscle tone and locomotor activity. Three doses of Anxy that did not affect these parameters were used. Locomotor activity was assessed with open field test (4). Concurrent control group of animals received appropriate volume of vehicle, 1% gum acacia solution. Drugs, dosage and number of animals used per treatment are shown in (table 1), (table 2), (table 3), (table 4), (table 5) and (table 6).

In acute study, drugs/vehicle was administered 60 min prior to experiment. In chronic study they were administered once daily for 10 days and the last dose was given on the 10th day, 60 min prior to experiment.

Apparatus

Elevated plus maze
The wooden maze consisted of two open arms (length 50 cm X breadth 10 cm) and two closed arms of the same size (height 40 cm). The arms of the same type were opposite to each other, with a central square of 10 cm. The maze was elevated to a height of 50 cm above the floor.

Light and dark box

The apparatus consisted of an open top wooden box. Two distinct chambers, a black chamber (20 X 30 X 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 X 30 X 35 cm) painted white and brightly illuminated with 100 W white light sources, were located 17 cm above the box. The two chambers were connected through a small open doorway (7.5 X 5 cm) situated on the floor level at the centre of the partition.

Open field apparatus

The apparatus consisted of a large rectangular box (100 X 80 cm) with 60 cm high walls. The floor was made of wire mesh and divided into twenty-five squares (outer 16 and central 9). The box was illuminated with 100 W bulb placed 60 cm above the centre of the field.

Behavioural assessment

Each animal was tested initially in plus maze and, then, in bright and dark arena paradigm in a single setting. In acute study 60 min after and in chronic study 60 min after the last dose on the 10th day of drug or vehicle administration, each animal was placed in the centre square of the plus maze, facing one of the open arms. The number of entries into and the time spent in open and closed arms and the number of rears in each arm in a five-minute period was noted.

Following the elevated plus maze test, the animal was placed at the centre of the brightly lit arena in the light and dark box. The number of entries into and the time spent in the bright arena, the number of rears in the bright and dark arenas and the duration of immobility were noted.

Following each trial, the apparatus were cleaned to mask the odour left by the animal in the previous experiment. Hand operated counters and stop watches were used to score the behaviour of animals.

In the open field test each animal was placed in one of the peripheral corner squares of the box and the number of peripheral and central squares crossed, the time spent in central squares and the number of rears were observed for a five-minute period.

Statistical analysis

The data were analysed by one-way ANOVA with drug treatment as the independent factor. Post-hoc comparisons were performed by applying Dunnett's test. $P < 0.05$ was considered statistically significant.

Results

Locomotor activity - Open field test

In the open field test, with acute drug treatment both the standard and the test drug did not increase the total number of squares crossed (table 1). Anxy at the highest dose (20 mg/kg), however, increased both the number of inner squares crossed and the time spent in them. Diazepam (1 mg/kg) increased only the time spent in inner squares without any significant change in crossing of inner squares.

Table 1: Effects of diazepam and Anxy on behaviour of rats in open field (acute study)

Treatment	n	Dose	Number of squares crossed			Time spent in central squares (sec)	Number of rears
			Peripheral	central	Total		
1% acacia	8	10 ml	59.62±5.5	3.75±0.94	63.37±4.83	3.50±1.21	6.75±0.90
Diazepam	6	0.5 mg	68.16±7.06	3.5±1.08	71.66±9.00	4.16±1.92	12.66±1.35
Diazepam	6	1 mg	59.66±7.06	6.5±1.14	66.16±7.32	9.33±2.78	11.83±1.70
Anxy	6	5 mg	69.5±9.15	2.83±1.36	72.16±10.13	1.83±0.79	14.16±2.89
Anxy	6	10 mg	61.7±7.02	5.16±1.62	66.33±8.38	5.33±1.38	18.83±1.62**
Anxy	6	20 mg	75.0±5.90	11.00±1.91	86.00±6.55	9.33±0.84*	22.16±4.06**
One-way		F	3.90			3.50	6.32
ANOVA		P	<0.01			<0.05	<0.01

Values are mean SEM. df =5, 32. *P<0.05, **P<0.001, compared to respective vehicle treated control. One-way ANOVA followed by Dunnett's test.

In the chronic study, both diazepam (0.5 mg/kg) and Anxy (5 and 10 mg/kg) not only increased the total number of squares (both peripheral and inner) crossed, but also the time spent in inner squares and the rears. At the highest dose (20 mg/kg), however, the test drug failed to alter any of the parameters studied (table 2).

Table 2: Effects of diazepam and Anxy on behaviour of rats in open field (chronic study)

Treatment	n	Dose	Number of squares crossed			Time spent in central squares (sec)	Number of rears
			Peripheral	central	Total		
1% acacia	8	10 ml	56.25±5.32	3.25±1.19	59.5±5.97	5.75±2.98	8.75±1.84
Diazepam	6	0.5 mg	82.16±3.42	10.0±2.26*	92.16±3.68*	14.0±1.98*	21.16±2.49**
Anxy	6	5 mg	85.00±1048*	10.66±1.68*	95.66±9.26*	8.83±1.70	19.00±3.17*
Anxy	6	10 mg	83.85±8.68*	12.33±1.96**	96.16±10.24*	14.33±1.82*	18.5±2.94*
Anxy	6	20 mg	65.16±7.26	4.66±1.54	69.83±8.24	6.83±1.51	13.83±2.75
One-way		F	3.48	5.68	5.21	3.77	4.04
ANOVA		P	<0.05	<0.05	<0.01	<0.05	<0.05

Values are mean SEM. df=4, 31. *P<0.05, **P<0.001, compared to respective vehicle treated control. One-way ANOVA followed by Dunnett's test.

Elevated plus maze

Acute treatment

Diazepam treated rats showed a significant increase in the number of open arm entries (1 mg/kg), percentile ratio of open arm to total arm entries (0.5 and 1 mg/kg), time spent in the open arms (1 mg/kg) and number of rears in the open arms (0.5 and 1 mg/kg). They showed a reduction in time spent in the closed arms (1 mg/kg).

Anxy treated rats exhibited a significant increase in the number of open arm entries (10 and 20 mg/kg), time spent in the open arms (5, 10 and 20 mg/kg), percentile ratio of open arm to

total arm entries (10 and 20 mg/kg), number of total arm entries (10 and 20 mg/kg) and number of rears in the open arms (20 mg/kg), but a decrease in time spent in the closed arms (20 mg/kg) (table 3).

Table 3: Effects of diazepam and Anxy on behaviour of rats in elevated plus maze (acute study)

Treatment	n	Dose	Number of arm entries		Percentile ratio of open/total arm entries	Time spent (sec)		Number of rears in open arms
			open	Total		Open arms	Closed arms	
1% acacia	8	10 ml	1.50±0.50	6.38±1.37	21.18±5.09	16.13±4.93	221.5±14.58	1.00±0.45
Diazepam	6	0.5 mg	3.67±0.42	8.17±0.83	44.53±1.34**	45.50±9.82	184.33±10.94	4.17±0.60**
Diazepam	6	1 mg	4.50±0.22*	10.50±0.67	43.37±2.42**	69.5±10.50*	158.83±10.56*	3.00±0.62*
Anxy	6	5 mg	2.33±0.80	7.00±1.57	31.33±4.71	67.17±21.09*	90.00±18.36**	1.00±0.67
Anxy	6	10 mg	5.17±1.62*	11.83±2.22*	40.73±4.39**	80.83±20.44*	72.33±13.93**	1.50±0.62
Anxy	6	20 mg	5.67±0.84**	14.17±1.33**	40.84±4.04**	117.33±17.89**	40.17±19.95**	3.16±0.47*
One-way		F	3.87	4.50	3.42	5.85	3.17	5.82
ANOVA		P	<0.05	<0.01	<0.05	<0.01	<0.01	<0.05

Values are mean SEM. df=5, 32. *P<0.05, **P<0.001, compared to respective vehicle treated control. One-way ANOVA followed by Dunnett's test.

Chronic treatment

Diazepam (0.5 mg/kg) significantly increased the number of entries into and the time spent in open arms, total arm entries and rears in open arms as well as the percentile ratio of open arm to total arm entries. The reduction in time spent in the closed arms was not significant. Repeated administration of Anxy significantly increased the open arm entries (2.5 and 5 mg/kg), time spent (2.5, 5 and 10 mg/kg), rears in open arms (2.5 and 5 mg/kg), total arm entries (2.5 and 5 mg/kg) and percentile ratio of open to total arm entries (2.5, 5 and 10 mg/kg). At all the doses tested, Anxy significantly reduced time spent in the closed arms (table 4).

Table 4: Effects of diazepam and Anxy on behaviour of rats in elevated plus maze (chronic study)

Treatment	n	Dose	Number of arm entries		Percentile ratio of open/total arm entries	Time spent (sec)		Number of rears in open arms
			open	Total		Open arms	Closed arms	
1% acacia	8	10 ml	1.62±0.49	5.50±10.5	24.58±5.94	30.75±12.70	207.87±21.87	1.87±0.63
Diazepam	6	0.5 mg	9.00±1.23**	13.66±1.11**	64.60±4.87**	101.00±6.05*	155.16±12.32	5.66±0.71*
Anxy	6	5 mg	6.83±1.35**	11.33±1.33**	57.71±5.89**	104.00±27.35*	117.83±20.06*	7.16±2.02**
Anxy	6	10 mg	7.33±1.22**	12.16±1.27**	63.24±6.59**	152.33±26.73**	96.93±21.84**	6.33±0.88*
Anxy	6	20 mg	3.66±0.66	6.83±0.30	55.80±8.56**	148.33±22.00**	134.83±22.22*	2.50±0.42
One-way		F	11.23	11.13	8.33	6.76	4.75	4.61
ANOVA		P	<0.01	<0.01	<0.01	<0.05	<0.05	<0.001

Values are mean SEM. df =4,27. *P<0.05, **P<0.001, compared to respective vehicle treated control. One-way ANOVA followed by Dunnett's test.

Light and dark box

Acute treatment

Diazepam treated rats significantly increased the time spent (0.5 mg/kg) and the rears (0.5 and 1 mg/kg) in light arena and decreased the duration of immobility (0.5 mg/kg). At both the doses (0.5 and 1 mg/kg), however, it did not alter the number of entries into bright chamber to any significant extent. Anxy treated rats showed a significant increase in the time spent (10 and 20 mg/kg) in light arena, while rearing (20 mg/kg) was increased in bright arena and dark arena. The test drug reduced the duration of immobility at the highest dose (20 mg/kg). Increase in the number of entries into light chamber was not significant (table 5).

Table 5: Effects of Anxy on behaviour of rats in bright and dark arena (acute study)

Treatment	n	Dose	Number of entries into light chambers	Time spent in light chamber(sec)	Number of rears in chamber		Duration of immobility(sec)
					Light	Dark	
1% acacia	8	10 ml	2.00±0.25	7.62±1.48	2.00±0.37	9.67±0.75	78.87±11.40
Diazepam	6	0.5 mg	3.33±0.42	40.83±8.80**	8.16±1.30**	15.50±2.26	14.66±5.44*
Diazepam	6	1 mg	2.16±0.40	19.66±7.80	5.66±0.71*	12.16±2.05	62.00±20.3
Anxy	6	5 mg	1.66±0.33	11.33±1.20	3.17±0.87	6.66±1.64	62.33±23.63
Anxy	6	10 mg	2.33±0.49	27.50±5.69*	3.83±0.79	16.33±2.55	59.5±23.61
Anxy	6	20mg	2.66±0.33	26.33±2.21*	5.16±1.01*	17.00±2.44*	9.83±4.13*
One-way		F	2.20	5.61	6.94	2.25	2.65
ANOVA		P	NS	<0.05	<0.01	<0.05	<0.05

Values are mean SEM. df=5, 23 *P<0.05, **P<0.001, compared to respective vehicle treated control. One-way ANOVA followed by Dunnett's test.

Chronic treatment

Repeated administration of diazepam (0.5 mg/kg) significantly increased the time spent and the rears in light arena and decreased the duration of immobility. It did not, however, alter the number of entries into light chamber to any significant extent. Anxy, on repeated administration, increased the number of entries (5 and 10 mg/kg), time spent (5, 10 and 20 mg/kg) and rears in light as well as in dark chambers (5, 10 and 20 mg/kg). It decreased the duration of immobility at all the doses (5, 10 and 20 mg/kg) tested (table 6).

Table 6: Effects of Anxy on behaviour of rats in bright and dark arena (chronic study)

Treatment	n	Dose	Number of entries into light chambers	Time spent in light chamber(sec)	Number of rears in chamber		Duration of immobility(sec)
					Light	Dark	
1% acacia	8	10 ml	1.39±0.18	13.12±2.05	2.7±0.65	5.75±0.95	164.12±21.42
Diazepam	6	0.5 mg	2.66±0.33	22.66±4.66*	6.83±1.07*	8.83±1.16	69.16±8.19**
Anxy	6	5 mg	2.83±0.50*	25.00±4.82*	7.33±0.88*	15.16±2.79**	11.33±5.48*
Anxy	6	10 mg	3.00±0.73*	36.66±10.04*	7.50±1.76**	20.66±3.96*	18.16±7.17*
Anxy	6	20mg	2.66±0.21	35.66±5.74*	6.50±0.84*	14.16±2.41*	6.66±3.53*
One-way		F	3.32	3.54	3.84	6.28	28.03
ANOVA		P	<0.05	<0.05	<0.05	<0.05	<0.01

Discussion

The two experimental models of anxiety, elevated plus maze and bright and dark arena are based on the assumption that unfamiliar, non-protective and brightly lit environmental stress provokes inhibition of normal behaviour. This normal behavioural inhibition is further augmented in the presence of fear or anxiety like state.

In the elevated plus maze, the open arms are more fear provoking than the closed arms. The ratio of entries, time spent and rearing behaviour in open arms to closed arms reflects the safety of closed arms with relative fearfulness of open arms (2).The reduction in entry, time spent, rearing in open arms, ratio of open arm to total arm entries and increased defecation are the indications of high level of fear or anxiety. Anxiolytic drugs increase the proportion of entries, time spent and rearing in open arms. They also increase the ratio of open arm to total arm entries.

In the light and dark box paradigm, the brightly lit environment is a noxious environment stressor that inhibits the exploratory behaviour of rodents. Reduction in the number of entries, time spent and rearing behaviour in the light chamber is regarded as markers of anxiety (3). Rearing reflects an exploratory tendency (5) of the animal that can be reduced due to a high level of fear.

In the present study, rats that received acute as well as chronic diazepam showed a significant increase in the time spent and the rears in open arms and the percentile ratio of open arms. They showed a decrease, however, in the time spent in closed arms of elevated plus maze. They also showed an increase in the time spent and the rears in bright arena and a decrease in the duration of immobility in the light and dark arena paradigm. All these suggest decreased fear, an increased exploratory behaviour and the behavioural disinhbitory effect of the standard anxiolytic. The test compound, Anxy, increased the number of entries, time spent and rearing in open arms and also increased the percentile ratio of open arm to total arm entries in the elevated plus maze paradigm. In light and dark paradigm, the test drug significantly increased the time spent in light arena, rears in both light and dark arena and transition between chambers. All these behavioural changes in both paradigms are suggestive of decreased fear, decreased aversion to bright light and increased exploratory behaviour of the animal. These behavioural changes produced by the test compound Anxy were comparable to those produced by diazepam.

The open field paradigm is used mainly to assess the locomotor activity of animals. In acute study, there was no significant change in the total number of squares crossed. This indicated the lack of effect of both the test and the standard drug on general motor activity. On repeated administration, however, the locomotor activity was increased as indicated by the enhanced peripheral, central and total number squares crossed. In addition, increased rearing, number of inner squares crossed and time spent in them reflect enhanced exploratory activity and reduced fear (6).

Withania somnifera, one of the constituents of Anxy, possesses antistress, adaptogenic and immunomodulatory properties, (7) with GABA mimetic activity (8), (9). Probably this antistress property contributes to the anxiolytic effect of Anxy. In addition, it has also been reported that the extract of *Withania somnifera* root and its bioactive compounds reduce the enhanced brain levels of tribulin, 5HT and corticotrophin, which are linked with anxiety state (8),(9),(10),(11).

Both *Withania somnifera* and *Ocimum sanctum* possess antistressor, adaptogenic, immunomodulatory and antioxidant properties. Ocimum sanctum is shown to have cortisol sparing, immunostimulant and antioxidant activities. This cortisol sparing immunomodulatory activity of *Ocimum sanctum* may also contribute to the behavioural disinhbitory activity (12), (13), (14).

Camellia sinensis, green tea, contains methyl xanthines (caffeine, theophylline), polyphenols and ascorbic acid. Methyl xanthines are central nervous system stimulants and have adenosine antagonistic activity. Adenosine agonists and antagonists produce anxiogenic and anxiolytic effects, respectively, in experimental animals (15). Therefore, the role of methylxanthines of *Camellia sinensis* in the overall anxiolytic activity of Anxy cannot be explained.

In folk medicine, *Sphaerunthus indicus* is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias (16). It is used to treat vitiated conditions of hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. It is also used as a nervine tonic. The oil prepared from the plant roots is reportedly useful in treating scrofula and as an aphrodisiac. The external application of a paste of this herb is beneficial in treating pruritus and edema, arthritis, filariasis, gout and cervical adenopathy. It also treats piles and hepatitis (17).

Although the polyherbal formulation, Anxy, has shown anxiolytic-like effect, it is difficult to point out which constituent is responsible for the same. Ayurvedic treatises claim that drugs should not be used alone and should be used in combination. Drug combination ensures synergism and helps to overcome the side effects of the other drug (18). This may be true for Anxy also, as this contains *Withania somnifera* extract that has sedative property (8). This sedative effect is ameliorated by bioactive of *Camellia sinensis* that has a stimulant effect (19).

As the test drug possesses anxiolytic-like effect similar to that of diazepam, it can be further studied to determine its possible use in human beings.

Acknowledgments

The authors are grateful to Srinivas Natural Remedies Pvt. Ltd, Mangalore for providing the Anxy formulation and A.Shama Rao Foundation for providing financial assistance.

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