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Original Article

Evaluation of anxiolytic activity of tensarin in mice

Rauniar GP[1], Deo S[2], Bhattacharya SK[3]
[1]Additional Professor [2]PG student [3]Professor and Head, Department of Pharmacology, BP Koirala Institute of Health Sciences, Ghopa, Dharan, Nepal

Abstract
Introduction: Anxiolytic drugs are amongst the most frequently prescribed drugs. Available anxiolytic agents are associated with several limitations. Several indigenous drugs are being evaluated but none has been proved to be effective.
Objectives: Aim of the present study is to evaluate the anxiolytic effect of Tensarin.
Material and method: The behavioural tests were conducted with single dose schedule and multiple seven-dose schedules of Tensarin 50mg/kg, 100mg/kg and 200mg/kg in comparison with Diazepam 1mg/kg in mice using open field test, activity-monitoring and passive avoidance test. There were eight treatment groups in each treatment schedule. Each group consisted of ten animals of either sex. The data obtained were analyzed using non- parametric test and P-value of less than 0.05 was considered to be statistically significant.
Results: Multiple doses produced anxiolytic effect as indicated by an increase in rearing, number of crossing and the time spent by the animals in Central Square. It was also seen that there was significant decrease in step down latency, increase in step down error and time spent by animal in shock zone, these effects were not observed in single dose study.
Conclusion: Tensarin shows a dose dependent anxiolytic effect but further studies are needed to find out the exact mechanism of action of the formulation.

Key words: Tensarin, Anxiolytic activity, Open field test, Passive avoidance test.

Anxiolytic drugs are amongst the most frequently prescribed drugs as anxiety is prevalent in the society. Existing anxiolytic agents are associated with several limitations such as sedation and addiction with benzodiazepines, tachycardia and ineffectiveness (in severe cases) with buspirone, insomnia, decreased libido and ineffectiveness (delayed but sustained) with fluoxetine,[1] which is an antidepressant having selective serotonin reuptake inhibitor (SSRI) property, being extensively used as well in patients of generalized anxiety disorder including social anxiety. These are some of the factors that led to the interest in using alternative remedies.

Several indigenous drugs are being evaluated because of their easy availability, lack of adverse effects and cost-effectiveness. Traditional medicines are used by about 60 percent of the world population in rural areas in the developing countries as well as in the developed countries where use of modern medicine predominates.[2] Though the use of herbal medicine is steadily increasing in western world,[3] the major hindrance in the amalgamation of herbal medicine into medical practice is the lack of sufficient scientific and clinical data and better understanding of efficacy and safety of the herbal products.[4] The historical use of such medicine provides the source to study the specific plant species with potential to be used in a particular disease.

Tensarin, a herbal preparation derived from Nepal's rich culture in traditional medicine[5], is a blend of various plant extracts such as Nordostachys Jatamansi (root) 100 mg, Rauwolfia serpentina (root) 100mg, Acorus Calamus (rhizome) 75 mg, Elaeocarpus Ganitrus (seed) 75 mg, Withania Somnifera (rhizome) 75 mg and Tinospora Cordifolia (stem) 75 mg and is claimed to have anxiolytic and sedative properties.[6] There is a need to establish the pharmacological activities of these ingredients for identifying and comparing the various components for potency and efficacy. Herbal products are often perceived as safe because they are natural,[7] though many dangerous and / lethal side effects have been reported from the use of these agents.[3]

Correspondence
Dr. G. P. Rauniar
Additional Professor and Program Coordinator MBBS Phase-I
Department of Pharmacology,
BP Koirala Institute of Health Sciences,
Ghopa, Dharan, Nepal
Email: gprauniar@hotmail.com

Risk of combining herbal products with branded drugs is mostly unknown. Adverse drug reaction monitoring of herbs may be very few because most patients do not discuss the use of herbal and other complementary medicines with their physician.[8] We believe that future investigations into the efficacy of herbal products should target both herbs with promising preliminary evidence of efficacy and conditions where standard medical therapies have failed to produce substantial benefits.

Hence, the aim of the present study is to evaluate the anxiolytic effect of Tensarin in comparison to a standard drug like Diazepam in mice using open field, activity-monitoring and passive avoidance test.

Research design and methodology:
This animal experimental study was carried out to evaluate the anxiolytic effect of Tensarin in mice in the department of Pharmacology, B. P. Koirala Institute of health sciences, Dharan Nepal after getting approval from the institutional research committee.

Maintenance of animals:
Ethics of animal experiments: Animals were maintained as per standard guidelines of the Indian National Science Academy (INSA) for the maintenance of laboratory animals.
Locally inbred albino mice of either sex (25-30g) were used. Mice were randomly assigned for experimental purpose and mice were housed in groups of 10 mice of same sex per cage in natural day and night cycle with maintenance of room temperature at near to 25 °c by air conditioner with free access to standard feed pellets (Kamadhenu mills, Dhulabari), soaked Bengal gram and water ad libitum. The animals were transferred to the laboratory at least one hour before the start of the experiment. The experiments were performed during day.

Preparation of drugs:
Tensarin tablets (Gorkha Ayurved Co. (P) Ltd , a Nepalese- French joint venture, post box:3666, Kathmandu, Nepal) were obtained from the market each tablet containing Nordostachys Jatamansi (root) 100 mg, Rauwolfia serpentina (root) 100mg, Acorus Calamus (rhizome) 75 mg, Elaeocarpus Ganitrus (seed) 75 mg, Withania Somnifera(rhizome) 75 mg and Tinospora Cordifolia (stem) 75 mg. Tablets were crushed to powder form. The powder was mixed with the 0.5 % Carboxy methyl cellulose (CMC) solution. The solution was administered orally with the help of an orogastric tube.

Diazepam tablets (Valium 5 mg, manufactured by Nicolas Piramal India limited, Pitampur- district, Madhyapradesh, Vallium trademark under licensed from F. Hoffman- La Roche limited, Basale Switzerland, TM Owner) were procured from market and were crushed to powder from with the help of mortar and pestle. The powder was suspended in 0.5% Carboxy Methyl cellulose (CMC).

Carboxy Methyl cellulose (CMC sodium salt, 500 gram, Batch No.-04078, product No.-027929, manufactured by Central Drug House (P) Limited, Bombay-New Delhi) was procured from market for use as vehicle for the preparation of suspension. On the study day, drugs were prepared fresh in the early morning using 0.5% Carboxy Methyl cellulose solution and were administered in a volume of 10 ml / kg orally with the help of an orogastric tube.

Study design: The behavioural tests were conducted with single dose schedule and multiple seven-dose schedules. There were eight treatment groups in each treatment schedule. Each group consists of ten animals of either sex. The mice were tested only once after the completion of the drug treatment schedule in the open field, passive avoidance apparatus and horizontal bar test. The study was carried out in a sound proof room and observations were made through an inner circuit television to avoid disturbances to the animals during the behavioural studies. Exposure to a novel environment is associated with emotional disturbance and anxiety. An anxious animal shows reduced ambulation associated with periodic freeze or immobility and reduction in normal behaviour such as rearing and grooming. Anxiety is also associated with augmented autonomic activity resulting in increased defecation and urination. All these effects are accentuated by anxiogenic drugs and attenuated by anxiolytics. Standard screening procedures such as locomotor activity, open field method and passive avoidance test are used to screen the anxiolytic effect of drugs in comparison with a standard drug like diazepam.[10] Simple open field model was used in our study that is more sensitive to anxiolytic effect produced by classical benzodiazepines and is effective in screening different classes of anxiogenic and anxiolytic agents.

Drug administration:
Single dose study:
Overnight fasted animals were selected randomly on the day of experiment for administration of vehicle, standard drug and study drug. The animals were acclimatized one hour before for behavioural tests. One hour time interval between drug administration and behavioural tests was maintained.

Multiple dose study:
Drug was administered orally to overnight fasted animals as single dose everyday in the morning for seven successive days and food was provided after one hour of drug administration so that food did not impair with the drug absorption but on the test day food was provided after completion of study. Behavioural tests were done after seven doses maintaining an interval of one hour for control, standard and study drugs after last dose.

Group of animals and doses of the drugs

S. N.	Group	No. of animals	Route of administration	Dose (mg/ kg)
1	CMC 0.5% (control)	10	oral	10 ml/kg
2	Diazepam (standard)	10	oral	1mg/kg
3	Tensarin 50	10	oral	50 mg/ kg
4	Tensarin 100	10	oral	100 mg / kg
5	Tensarin 200	10	oral	200 mg / kg
6	Tensarin 50+Diazepam	10	oral	50mg/kg
7	Tensarin 100+Diazepam	10	oral	100mg/kg + 1mg/kg
8	Tensarin 200+Diazepam	10	oral	200mg/kg + 1mg/kg

- *Diazepam was used 1mg/kg either alone or in combination with different doses of Tensarin*
- *Tensarin was used 50mg/kg, 100mg/kg, 200mg/kg either alone or in combination with Diazepam.*

Experimental procedures:
Open field:
This test utilizes behavioural changes in rodents exposed to novel environments and is used to detect angiogenic and anxiolytic activity under identical situations. Various types of Open field apparatus have been used to test the mice.
An 'open field apparatus'; suitable for mice were made comprising of a floor space of 40 cm. x 40 cm with 30 cm. high walls. The floor was coloured black and the floor area was divided into 9 equal squares by white lines. A mouse was placed at the center[9] of the field and was left for 2 minutes for acclimatization with the apparatus. Thereafter, for the next 5 min., the following parameters were noted:

a) Time spent in the central square
b) Ambulation (No. of squares crossed)
c) Rearing (No. of times the animal stands on the rear paws)

Passive avoidance
This test is done in a chamber of the size 34cm× 34cm × 20cm with a grid floor through which electric shock of 20 mv was delivered. A shock free zone (SFZ) is provided in the centre of the chamber by placing an inverted petri dish. Mice were placed on the SFZ and when they try to get down from the SFZ and come in contact with the grid floor, they received electric shock. Mice gradually learnt to avoid shock by staying in the SFZ curbing their normal exploratory behaviour. This is the principle of passive avoidance test. The animal was initially trained till it avoid coming in contact with the shock zone by passively sitting on the SFZ for at least a minimum of 60 sec. Those mice, which did not learn in 5 training sessions, were discarded.
The parameters noted were:

1. Step down latency (duration for which the animal stayed in the SFZ)
2. Step down error (number of attempts the animal made to come to the shock zone)
3. Total time spent in the shock zone

Statistical analysis: The data obtained were analyzed by using Student's t test and Wilcoxon signed ranks test using SPSS 10.5 and Microsoft excel 2002 versions. P value <0.05 was considered statistically significant.

Results:
Number of squares crossed
There was a significant increase in the number of square crossed in diazepam group compared to control. Though there was slight increase in the number of squares crossed by mice in Tensarin treated groups (50, 100, 200 mg/kg) as compared to control, it was not statistically significant but when Diazepam (1 mg /kg) was co-administered with identical doses of Tensarin, the increase in the number of squares crossed by mice was statistically significant as compared to the standard (Table-1).

Time spent in Central Square

Time spent in Central Square in the control and standard (Diazepam) group were 1.7 ± 1.42 and 7.5±4.81 sec respectively. There was a significant increase in diazepam group as compared to control group. But when different doses of Tensarin were used alone the increase in time spent in central square was not statistically significant. However, when Diazepam (1 mg/kg) was combined with different doses of Tensarin, there was statistically significant increase in time spent in Central Square by the animal. (Table 1)

Number of rearing

There was significant increase in the rearing of animals with diazepam in comparison to the control group. There was also increased number of rearing in test drug plus diazepam group which was not statistically significant (Table1).

Step down latency

There was significant prolongation of the step down latency in standard group as compared to control group. There was no significant difference between the control and other treatment groups (Table2).

Step down Error

A significant increase in step down error in standard group was observed as compared to the control group but there was no statistical difference in other treatment groups compared to standard (Table2)

Time spent in the shock zone

There was a significant increase in the time spent in the shock zone with standard treated group compared to control (Table2)

Single dose schedule of Tensarin did not produce significant changes in animal behavioral parameter of open field and passive avoidance test.

Open field test

Number of squares crossed

There was significant increase in number of squares crossed in the diazepam treated group and also in T100 as well as in T200 treated group as compared to control. There was also an increase in number of square crossed in T100+D and T200+ D groups which was statistically significant when compared with standard group (Table3).

Time spent in the central Square

There was a significant increase in Time spent in Central Square in standard as well as in T200 group as compared with the control. There was no statistically significant difference in other parameters (Table 3).

Number of rearing

Number of rearing was increased significantly in standard group and also in T100 as well as in T200 groups as compared to the control group. With T200+D, there was also a significant increase in number of rearing when compared to standard group (Table 3).

Step down latency

There was statistically significant difference found between control and T100 as well as T200 test group. There was also decrease in latency time in T200+D group as compared with the standard (Table4).

Step down error

There was increase in step down error which was statistically significant in standard and T100 groups as compared to control group. In T200+D group, significant increase in step down error when compared to standard group (Table 4).

Time spent in the shock zone

Time spent in shock zone was increased in diazepam, T100 and T200 in comparison to control group. There were no differences in rest of the parameters (Table 4). When test dose was given for seven days, it was observed that there was increase in number of squares crossed, increase in Central time spent, number of rearing was increased in open field behavioral model and decrease in latency time, increase in step down error, and increased time spent in shock zone was seen in passive avoidance model.

Single dose Schedule

Table1. Effect of single dose observation in open field test

S. N.	Group	Mean ± (SD)			
		No. of animals	No. of Square	Time spent in central square	No. of Rearing
1	Control	10	84.8 (10.21)	1.7 (1.42)	17.2 (9.21)
2	Diazepam	10	164.5 (44.75)*	7.5 (4.81)*	32.2 (11.21)*
3	Tensarin 50	10	90.6 (21.56)	1.8 (1.62)	18.3 (6.85)
4	Tensarin 100	10	91.7 (16.38)	2.3 (1.89)	21.7 (7.47)
5	Tensarin 200	10	95.3 (29.41)	2.9 (1.91)	26.3 (7.94)
6	Tensarin 50+Diazepam	10	153.7 (30.01) *	14.1 (6.38) *	34.4 (11.20)
7	Tensarin 100+Diazepam	10	178.9 (46.26) *	16.8 (14.23)*	44.3 (11.62)*
8	Tensarin 200+Diazepam	10	203.9 (77.23)*♣	25.3 (16.47)*	49.3 (14.18)*

**Significant P<0.05 compared to control group.*

♣ Significant P <0.05 compared to Standard group.

Passive avoidance test

Table2: Effect of single dose observation in passive avoidance test

S. N.	Group	Mean ± (SD)			
		No. of animals	Step down latency (second)	Step down error	Time in shock zone
1	Control	10	230.3 (54.83)	1.3 (0.95)	9.6 (5.70)
	Diazepam	10	85.6 (81.80)*	6.2 (4.08)*	41 (16.80)*
	Tensarin 50	10	233.6 (49.94)	1.9 (1.02)	11.4 (4.22)
	Tensarin 100	10	252.6 (57.09)	1.8 (1.62)	12.5 (4.53)
	Tensarin 200	10	219.3 (72.29)	2.2 (2.04)	13 (4.24)
	Tensarin 50+Diazepam	10	171.4 (80.71)	6.9 (3.11) *	58.8 (48.30) *
	Tensarin 100+Diazepam	10	150.9 (99.68)	9.5 (7.93) *	61.7 (41.46) *
	Tensarin 200+Diazepam	10	46.4 (59.31) *	11.5 (6.24) *♣	72.1 (49.95) *

*Significant P<0.05 compared to control group.

♣ Significant P <0.05 compared to Standard group.

Multiple dose schedules

Table 3: Effect of multiple dose observation in open field test

S. N.	Group	Mean ± (SD)			
		No. of animals	No. of Square	Time spent in central square	No. of Rearing
1	Control	10	62.5 (12.37)	2.1(1.91)	13 (4.08)
	Diazepam	10	100.8 (21.99)*	13.1 (4.84)*	24.3 (4.40)*
	Tensarin 50	10	76.3 (14.61)	5.7 (4.42)	17.30 (5.21)
	Tensarin 100	10	87.9 (13.92)*	10.9 (10.42)	19.1 (4.58)*
	Tensarin 200	10	91.4 (23.23)*	10.2 (3.26)*	20.7 (5.23)*
	Tensarin 50+Diazepam	10	123.5 (17.80) *	13.1 (3.03) *	26.4 (7.11) *
	Tensarin 100+Diazepam	10	134.20 (17.41)* ♣	14.40 (2.55) *	29.40 (10.88) *
	Tensarin 200+Diazepam	10	151.90 (24.17)* ♣	18.80 (5.43) *♣	37.00 (12.89)*♣

** Significant P<0.05 compared to control group.*

♣ Significant P <0.05 compared to Standard group.

Passive avoidance test:

Table4: Effect of multiple dose observation in passive avoidance test

S. N.	Group	Mean ± (SD)			
		No. of animals	Step down latency (second)	Step down error	Time in shock zone
1	Control	10	269 (41.75)	0.7 (0.82)	4.4 (3.34)
2	Diazepam	10	161.3 (117.91)	4.4 (3.60)*	14.2 (2.57)*
3	Tensarin 50	10	166.5 (117.68)	2.3 (2.16)	8.4 (3.10)
4	Tensarin 100	10	195.6 (124.17)*	2.2 (2.44)*	12.7 (4.64)*
5	Tensarin 200	10	198.3 (120.90)*	2.6 (2.01)	14.2 (2.86)*
6	Tensarin 50+Diazepam	10	102.6 (77.07)*	6.3 (2.36)*	28.4 (17.58) *
7	Tensarin 100+Diazepam	10	73.1 (94.19)*	8.8 (3.08)*	42.2 (35.23)*♣
8	Tensarin 200+Diazepam	10	36.90 (77.50)* ♣	10.20 (3.68)*♣	63.5 (55.23)*

**Significant P<0.05 compared to control group.*

♣ Significant P <0.05 compared to Standard group.

Discussion

The incidence of pathologic anxiety in the community is very high and is associated with lot of morbidity. Lifetime prevalence in women is 30.5% and in males is 19.2%[10]. Hence, it is very important to address the problem of anxiety and find effective remedies. Though several drugs are available, all are associated with some limitations. Benzodiazepines are the standard anti-anxiety drugs but they are associated with problems of sedation and addiction. Buspirone, the non-sedative anxiolytic agent is not effective in a high percentage of patients. It is also associated with tachycardia, palpitation, gastric discomfort etc.[11] Refractory cases of anxiety need newer drugs, which are safe and without many side effects. There is an urgent need for alternative medications for anxiety.

Tensarin is a herbal medication with a combination of different ingredients like Nordostachys Jatamansi, Rauwolfia serpentina, Acorus Calamus, Elaeocarpus Ganitrus, Withania Somnifera and Tinospora Cordifolia. It has been reported to possess anxiolytic activity. According to the ayurveda, Nordostachys Jatamansi[6] have tranquilizing properties (100 mg/kg)[15] and has been used either alone or in-combination with other herbal drugs in relieving anxiety. It is also mentioned that Acorus Calamus[6] possess antianxiety properties (50 mg/kg I.P.) and controls anxiety, improves memory and hyperactivity in children,[12] and it may have anxiolytic action and also exerts sedative and tranquilizing action.[13] Tinospora Cordifolia[6] is considered as a nervine tonic to promote mental clarity, and it possesses anti-stress[14] (100mg/kg) properties. It produced a significant improvement in sleep parameter and had good hypnotic activity.[15] Withania Somnifera[6] is highly valuable for its restorative actions on the functions of nervous system. It restores the vitality in those suffering from over work and nervous exhaustion. In fact Withania Somnifera (100 mg/kg oral) has ability to control anxiety, stress and promote calm state of mind. The animal research and clinical trials also support the use of Withania Somnifera for anxiety[16] and its mood stabilizing effect in clinical conditions of anxiety depression[17] and has a potent antistress activity.[18] Elaeocarpus Ganitrus[6] (50-100 mg/kg I.P) is used in mental diseases as mentioned in ayurveda which is supported by Bhattacharya et al[19] and Rauwolfia serpentina alkaloid (100 mg/kg oral) interacts directly at benzodiazepine sites with benzodiazepine type activity[20].

In our study, Tensarin (50-200 mg/kg) given for 7 days wee observed to produce anxiolytic effects as indicated by an increase in rearing, number of crossing and time spent by animal in central square and it was also seen that there was significant decrease in step down latency, increase in step down error and time spent by animal in shock zone. It shows that Tensarin has some psychotropic effect in a dose dependent manner. Further studies are needed to find out the exact mechanism of action of the formulation.

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