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To Evaluate the Anxiolytic Effect of Diltiazem in Albino Rats by Elevated-Plus Maze Model.

KEYWORDS: Anxiolytic, Diltiazem, Potentiation.

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ABSTRACT Diltiazem, a Calcium channel antagonist (CCA), is widely used in treatment of variety of cardio-vascular disorders for their actions on the Voltage Sensitive Calcium Channels (VSCC) in cardiac and smooth muscles. As some patients with ischemic heart disease (IHD) have an anxiety state apart from chest pain and diltiazem is a known CCA prescribed for IHD patients, the present study is taken up to evaluate anxiolytic action of diltiazem in albino rats in elevated plus maze model, an experimental model of anxiety. Diltiazem is evaluated for anxiolytic effect in graded doses of 5mg/kg, 10mg/kg & 20mg/kg body wt i.p. (intraperitoneal) and their effects are compared with standard drug Diazepam. The test drug diltiazem produced significant anxiolytic effect at doses of 10mg/kg & 20mg/kg body wt i.p treatment. Further, low dose combination of Diltiazem 5 mg/kg body wt + Diazepam 1 mg/kg body wt i.p. treatment showed potentiation of anxiolytic effect in elevated plus maze model.

INTRODUCTION

Anxiety is a diffuse, highly unpleasant, vague feeling of apprehension accompanied by one or more bodily sensation (Benzamine JS *et. al.,* 2009). Anxiety disorders, the most prevalent psychiatric illnesses in the general community, are present in 15–20% of medical clinic patients (Dennis L *et. al.,* 2012).

Pharmacotherapeutic approaches for treatment of anxiety commonly include use of benzodiazepines but regular use of these causes deterioration of cognitive functioning, addiction, psychomotor impairment, confusion, aggression, excitement, anterograde amnesia, physical dependence and tolerance (Suresh K *et. al.,* 2006). However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects (Griffiths RR *et. al.,* 1987).

Calcium is one of the most important second messengers, and plays a major role in many of the neuropsychiatry disorders; especially mood disorders (Balon R *et. al.,* 1996). Calcium channel antagonists (CCAs) are widely used in treatment of variety of cardio-vascular disorders for their actions on the Voltage Sensitive Calcium Channels in cardiac and smooth muscles (Krishna HNG *et al.,* 2001). The CCA binding sites recognized in the limbic regions of brain has raised the hope that CCAs like Diltiazem might also be useful in some behavioral disorders (Krishna HNG *et al.,* 2001). As some patients with ischemic heart disease (IHD) have an anxiety state apart from chest pain and diltiazem is a known CCA prescribed for IHD patients, the present study is taken up to evaluate anxiolytic action of diltiazem in albino rats in Elevated-Plus Maze model, an experimental model of anxiety.

MATERIALS AND METHODS:

This study was undertaken to evaluate the anxiolytic activity of Diltiazem in graded doses (5, 10, 20 mg/kg; i.p), Diazepam (1, 2 mg/kg; i.p) and their low dose combination in Elevated-plus maze model of anxiety in Albino Rats. It was conducted in the department of Pharmacology, Kamineni Institute of Medical Sciences from September 2012 – August 2013.The study was randomized controlled based on laboratory animal model with permission of Institutional Animal Ethics Committee (IAEC).

I) Animals:

Wistar Albino Rats of either sex weighing 100-200 grams, bred in the Central Animal House, procured from National Institute of Nutrition, Hyderabad were used. The animals housed under standard laboratory conditions, maintained on 12:12 light dark cycle and had free access to food and water. The procedure is carried out in a sound attenuated room. The rats are housed in pairs for 10 days prior to testing.

The animals were acclimatized to laboratory conditions 1 week before the test. Number of animals required - 42(+7); Number of groups - 7; Number of animals in each group - 6.

ii) Drugs: Diazepam (Inj. Calmpose, Ranbaxy lab.) & Diltiazem (Inj. Dilzem, torrent pharma Limited)

iii) Grouping of Animals: (N = 42)

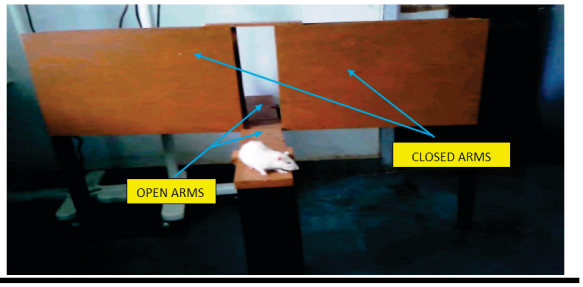
Grouping of Rats (n=6): Elevated Plus Maze Model.

Group I: Normal Saline (Control); 5ml/kg body wt i.p.
Group II: Diazepam (standard); 1mg/kg body wt i.p.
Group III: Diazepam (standard); 2mg/kg body wt i.p.
Group IV: Diltiazem (test drug); 5mg/kg body wt i.p.
Group V: Diltiazem (test drug); 10mg/kg body wt i.p.
Group VI: Diltiazem (test drug); 20mg/kg body wt i.p.
Group VII: Diltiazem + Diazepam (low doses); 5mg+1mg/kg body wt i.p.

iv) Experimental Design and Drug Treatment:

Elevated Plus Maze Model (Vogel HG, 2002) (Medhi B et. al., 2010): Description of Model: Elevated plus maze apparatus consists of 2 open and 2 closed arms with dimensions 50 x 10 cms and 50 x 10 x 40 cm respectively. It has an open roof. Entire maze is elevated 50 cms from floor.

Fig. No. 1. Showing Elevated Plus Maze Model:



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Procedure:

- Initially drug is given i.p. and after 30 min, rat will be placed individually at the center of maze with head facing towards an open arm and allowed to move for 5 min (300 sec) (Poonam mahendra *et al.,* 2011). In animals belonging to Group VII (combination), Diltiazem will be administered initially, followed by Diazepam after 30 min. i.e. parameters will be noted 1 hr after Diltiazem administration (Kozlovskii VL, 1999) (Hiremath *et al.,* 2010).

- The parameters recorded are **Time spent in open arms & closed arms, Number of entries into open arms & closed arms.**

- **Normally, rats (rodents) prefer to be in closed arms, but after anxiolytic drug administration they prefer more time in open arms**.

v) Statistical Analysis:

- Statistical Analysis is done by SPSS software version 19. Data is analyzed by using Analysis of Variance (ANOVA) with drug treatment as independent factor. p value < 0.05 is considered as statistically significant. Post-hoc comparisons are made using Least Significant Difference (LSD) test.

RESULTS:

The results of Elevated-plus maze model indicated a significant increase in time spent in open arm, significant decrease in time spent in closed arm and significant increase in number of entries into open arm in the i.p. treatment groups of Diazepam 2 mg/kg; Diltiazem 10mg/kg; Diltiazem 20mg/kg body wt compared to control group(NS) suggesting anti-anxiety action. Further combination of low dose diltiazem & diazepam showed potentiation by increasing time spent in open arm, decreasing time spent in closed arm and increasing number of entries into open arm in comparison to control group. The results given are Mean ± SEM. Refer to table no. 1 & 2.

Table No.1 Comparison of results of different groups in Elevated Plus Maze model (N=42)

Group (n=6) in each group	Drug	Dose in mg/kg(i.p.)	Time spent in open arm	Time spent in closed arm	Number of entries into open arm	Number of entries into closed arm
			Out of 300 sec		In 300 seconds	
I control	NS	0.5 ml	47.66 ± 2.19	252.33±2.11	1.17±0.38	3.33±0.33
II std	Diazepam	1	56.00 ± 3.88	244.0 ± 3.88	1.33±0.21	4.33±0.33
III std	Diazepam	2	122.50±9.04**	177.5±9.04**	3.83±0.36**	5.33±0.61**
IV test	Diltiazem	5	51.00 ± 2.21	249.0 ± 2.2	1.00 ± 0.26	3.0 ± 0.26
V test	Diltiazem	10	113.33 ± 3.6**	185.33±4.34**	3.5 ± 0.23**	5.0 ± 0.51*
VI test	Diltiazem	20	100.67±3.84**	199.33±3.84**	3.33±0.42**	4.5 ± 0.34*
VII combination	Diltiazem + Diazepam	5 + 1	78.67 ± 2.98**	221.33±2.98**	3.0 ± 0.36**	4.16 ± 0.31

Note: All values are in Mean ± SEM * p < 0.05 & ** p < 0.001 in comparison to control group.

Table No. 2 INTERGROUP COMPARISON BY ONE WAY ANOVA TEST

		Sum of Squares	Df	Mean Square	F	Sig.
Time Spent in Open Arm.	Between Groups	34769.28	6	5794.88	47.357	0.001**
	Within Groups	4282.83	35	122.36		
	Total	39052.11	41			
Time Spent in Closed Arm.	Between Groups	35289.28	6	5881.54	46.16	0.001**
	Within Groups	4458.83	35	127.39		
	Total	39748.11	41			
No. of entries into Open Arm.	Between Groups	54.57	6	9.09	16.05	0.001**
	Within Groups	19.83	35	0.57		
	Total	74.4	41			
No. of entries into Closed Arm.z	Between Groups	25.26	6	4.21	4.29	0.002*
	Within Groups	34.33	35	0.98		
	Total	59.61	41			

*** p < 0.05 (significant) & ** p < 0.001 (highly significant)**

DISCUSSION AND CONCLUSION:

Gopala Krishna *et. al.,* 2001 also studied the effect of diltiazem in elevated plus maze method in rats.

The results of present study with the diltiazem 10 mg/kg & 20 mg/kg on the parameters like time spent in open arm & no. of entries into open arm are similar as Gopala Krishna *et. al.* also observed increase in time spent in open arm & increase in no. of entries into open arm by diltiazem 10 mg/kg & 20 mg/kg. Refer to table no.3.

Table no. 3. Comparison of Results of Present Study with H. N. Gopala Krishna et al. Study 2001 in Elevated Plus Maze Method:

All drugs are administered by intraperitoneal route. (i.p.)		time spent in open arm out of 300 sec		No. of entries into open arm in 300 sec	
Drug	Dose (i.p.)	Present Study	H.N.Gopala krishna study 2001	Present Study	H.N.Gopala krishna study 2001
NS	1ml/kg	47.66 ± 2.19	5.73 ± 2.4	1.17 ± 0.38	0.83 ± 0.24
Diazepam	1mg/kg	56.00 ± 3.88	24.12±3.84*	1.33 ± 0.21	2.62 ± 0.67*
Diazepam	2mg/kg	122.5±9.04**		3.83±0.36**	
Diltiazem	5mg/kg	51.00 ± 2.21	14.22 ± 3.21	1.00 ± 0.26	1.25 ± 0.31
Diltiazem	10mg/kg	113.33 ± 3.6**	30.87 ± 8.1*	3.5 ± 0.23**	2.25 ± 0.55*
Diltiazem	20mg/kg	100.67±3.84**	19.59±6.15*	3.33±0.42**	2.00 ± 0.46*

The finding that L-type calcium channels found in amygdala play an important role in cued conditioning suggests that calcium is an important mediator of anxiety (Shinnick-Gallagher P *et al.,* 2003). The possible mechanism involved in anti-anxiety action of diltiazem could not only be due to primary action, inhibition of calcium influx, but also due to interactions with serotonergic receptors (Boullin DJ *et al.,* 1987) (Green AR *et al.,* 1990), dopaminergic receptors (Pucilowski O, 1992) (Czyrak A *et al.*, 1989), increased adenosine concentration at synaptic sites (Moron MA *et al.,* 1990) and decreased release of corticotropin releasing factor (Smith MA *et al.,* 1997).

The possible reason for potentiating action of diltiazem to anti-anxiety effect of diazepam might be due to different mechanisms of action of these two drugs.

Adinaik et al 2009 reported decreased number of entries into closed arm in elevated plus maze model with diazepam 2mg in comparison

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to control whereas in the present study diazepam 2 mg/kg treatment increased the number of entries into closed arm in comparison to control group. Reason for the difference in these two studies might be due to lab. to lab. variation or strain variation.

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