



Anxiolytic Effect of Diltiazem in Albino Rats by Light & Dark Arena Model.

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ABSTRACT

The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light. The aim of the present study was to explore the anti-anxiety activity of Diltiazem, a Calcium channel antagonist (CCA). Diltiazem is evaluated for anxiolytic effect in graded doses of 5mg/kg, 10mg/kg & 20mg/kg body wt i.p. 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 light & dark arena model.

KEYWORDS : Anxiolytic, Diltiazem, Diazepam.

INTRODUCTION

Anxiety is a diffuse, highly unpleasant, vague feeling of apprehension accompanied by one or more bodily sensation (Benzamine JS *et. al.*, 2009). Anxiety is a normal human emotion. Anxiety is considered excessive or pathological (JK Trivedi *et. al.*, 2010) (i)when it arises in absence of stress or challenge, (ii)when it is out of proportion to the challenge or stress in severity or duration, (iii)when it results in significant distress and (iv)when it results in psychological, social, occupational, biological and other impairment.

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). Calcium channel antagonists (CCAs) are widely used in treatment of variety of cardio-vascular 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 the anxiolytic action of diltiazem in albino rats in Light & dark arena model.

MATERIALS AND METHODS:

The study was randomized controlled based on laboratory animal model with permission of Institutional Animal Ethics Committee (IAEC). It was conducted in the department of Pharmacology, Kamineni Institute of Medical Sciences from September 2013 to August 2014. This was done 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 Light & dark arena model of anxiety in Albino Rats.

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 were acclimatized to laboratory conditions before the test and had free access to food & water. The procedure is carried out in a sound attenuated room. Experiment is done during light period between 9am & 1pm. 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:

Light And Dark Arena Model (Vogel HG, 2002)

Description of Model: The Apparatus consists of a box (44 x 21 x 21 cm) with two chambers (DARK & LIGHT) separated by a partition wall having a small door in its centre. The dark chamber constitutes nearly 1/3 of the total box and it is painted black inside & the roof is covered. The light chamber is transparent and well illuminated without roof.

Procedure:

- Initially the drug is given i.p. and after 30 min, rat will be placed individually in the center of light arena and will be allowed to move for 10 min (600 sec). 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 light & dark arena, Number of entries into light & dark arena.**
- Rats are rodents, feel safe in dark and hence normally rats prefer dark arena more. But after anxiolytic drug administration, they prefer light arena more.**

v) **Statistical Analysis:** Data is analyzed by using Analysis of Variance (ANOVA) with drug treatment as independent factor. p value < 0.05 is considered as statistically significant.

RESULTS:

The results of Light and Dark model indicated a significant increase in time spent in light arena, significant decrease in time spent in dark arena and significant increase in number of entries into light arena 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 light arena, decreasing time spent in dark arena and increasing number of entries into light arena in comparison to control group. The results given are Mean \pm SEM. Refer to table no. 1 & 2.

Table No.1 Comparison of results of different groups in Light and Dark Arena model (N=42)

Group (n=6) in each group	Drug	Dose in mg/kg (i.p.)	Time spent in light arena	Time spent in dark arena	Number of entries into light arena	Number of entries into dark arena
			Out of 600 sec		In 600 seconds	
I control	NS	0.5 ml	120.33 \pm 3.89	479.67 \pm 3.89	1.5 \pm 0.22	1.67 \pm 0.21
II std	Diazepam	1	123.12 \pm 2.44	476.83 \pm 2.44	1.66 \pm 0.21	2.0 \pm 0.0
III std	Diazepam	2	212.0 \pm 7.7**	388 \pm 7.7**	4.0 \pm 0.36 **	5.0 \pm 0.36**
IV test	Diltiazem	5	111.5 \pm 4.23	488.5 \pm 4.23	1.33 \pm 0.21	1.83 \pm 0.17
V test	Diltiazem	10	203.83 \pm 4.23**	396.16 \pm 4.23**	3.33 \pm 0.21**	4.33 \pm 0.21**
VI test	Diltiazem	20	179.5 \pm 3.62**	420.5 \pm 3.62**	3.0 \pm 0.26**	3.83 \pm 0.31**
VII combination	Diltiazem + Diazepam	5 + 1	179.67 \pm 5.51**	420.33 \pm 5.51**	2.67 \pm 0.21*	3.67 \pm 0.21**

Note: All values are in Mean \pm 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 Light arena	Between Groups	63962.952	6	10660.492	77.841	0.001**
	Within Groups	4793.333	35	136.952		
	Total	68756.286	41			
Time Spent in Dark arena	Between Groups	63962.952	6	10660.492	77.841	0.001**
	Within Groups	4793.333	35	136.952		
	Total	68756.286	41			
No. of entries into Light arena	Between Groups	37.667	6	6.278	17.121	0.001**
	Within Groups	12.833	35	0.367		
	Total	50.500	41			
No. of entries into Dark arena	Between Groups	64.810	6	10.802	32.405	0.001**
	Within Groups	11.667	35	0.333		
	Total	76.476	41			

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

DISCUSSION AND CONCLUSION:

The results of present study with the diltiazem 10 mg/kg & 20 mg/kg on the parameters like time spent in light arena & no. of entries into light arena are similar as Gopala Krishna *et al.* 2001 also observed increase in time spent in light arena & increase in no. of entries into

light arena 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 Light and Dark arena model:

All drugs are administered i.p.		time spent in light arena out of 600 sec		No. of entries into light arena in 600 sec	
Drug	Dose (i.p.)	Present Study	H.N.Gopala krishna study 2001	Present Study	H.N.Gopala krishna study 2001
NS	1ml/kg	120.33 \pm 3.89	29.58 \pm 5.22	1.5 \pm 0.22	1.5 \pm 0.19
Diazepam	1mg/kg	123.12 \pm 2.44	82.74 \pm 12.9*	1.66 \pm 0.21	2.37 \pm 0.32
Diazepam	2mg/kg	212.0 \pm 7.7**		4.0 \pm 0.36 **	
Diltiazem	5mg/kg	111.5 \pm 4.23	65.7 \pm 19.44	1.33 \pm 0.21	2.37 \pm 0.37
Diltiazem	10mg/kg	203.83 \pm 4.23**	85.98 \pm 16.44*	3.33 \pm 0.21*	3.00 \pm 0.56*
Diltiazem	20mg/kg	179.5 \pm 3.62**	106.98 \pm 18.7*	3.0 \pm 0.26**	3.00 \pm 0.46*

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).

Adinaik *et al* 2009 reported decreased number of entries into dark arena with diazepam 2mg in comparison to control whereas in the present study diazepam 2 mg/kg treatment increased the number of entries into dark arena in comparison to control group. Reason for the difference in these two studies might be due to lab. variation or strain variation.

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