ANTI-ANXIETY, ANTI-DEPRESSANT AND ANTI- CATALEPTIC ACTIVITY OF 4-HYDROXY-3-[2-(3-NITROPHENYL)-2, 3-DIHYDRO-1, 5-BENZOTHIAZEPIN-4-YL]-2H-CHROMEN-2-ONE

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Summary

The Present study was aimed to evaluate the therapeutic effectiveness of 1, 5-benzothiazapine derivative in central nervous diseases. 1,5- Benzothiazipines are the seven–membered heterocyclic ring systems which are reported for their behavioral activities. In the current research, we have investigated the effect of 1,5-benzothiazine derivative for its anti-anxiety, anti-depressant and anti-cataleptic activities by using elevated plus maze, forced swim test and chlorpromazine induced catalepsy respectively. Wistar rats were treated with 1, 5-benzothiazapine derivative (5mg/kg, p.o. daily) for 30 days. The results showed significant ($p<0.01$) anti-anxiety, anti-depressant and anti-cataleptic actions of 1, 5-benzothiazapine derivative. Here we suggested that, these drugs can be further studied in Preclinical and Clinical level for the treatment of central nervous diseases.

Keywords: Anxiety, Depression, Catalepsy, 1, 5 Benzothiazipines, Behavioral studies.

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Introduction

According to World Health Organization (WHO), neurological disorders like anxiety and depression affect up to one billion people worldwide. Neurological disorders also include brain injuries, neuro-infections, and multiple sclerosis and Parkinson disease. The most common among the neurological disorders are anxiety, depression and catalepsy.

Anxiety [1, 2] is an unpleasant, emotional state that involves a complex combination of emotions that include fear, apprehension, and worry. A mental and emotional state characterized by fear and uneasiness. Anxiety correlates highly with "stress". Depression [1, 3] is the most common of the affective disorders; it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. Worldwide depression is a major cause of disability and premature death. In addition to the suicide risk, depressed individuals are more likely to die from other causes, such as heart disease or cancer. Catalepsy [4] is a nervous disorder characterized by immobility and muscular rigidity, along with a decreased sensitivity to pain. In hypnosis, catalepsy refers to a state of muscular rigidity, usually of a part of the body, such as an arm, induced by the hypnotist in the patient. As a nervous condition, catalepsy is a symptom of a number of disorders, including Parkinson's disease, epilepsy, schizophrenia, cocaine withdrawal, sleep, apnea, obesity, depression, and emotional shock.

Benzothiazepines [5] play a unique role in drug discovery programs. They display a wide spectrum of biological activities such as anti anxiety, anti depressant and anti-cataleptic e.t.c. We have been interested in 1, 5-benzothiazepine derivatives and have prepared and evaluated its central nervous actions in experimental animals.

Materials and Methods

Animals:

Healthy, adult Wistar rats of both sexes (180-220g) were obtained from the Central animal house facility from J.S.S College of Pharmacy, Ootacamund, India and maintained under standard laboratory conditions. The experimental protocol was approved from Institutional Animal Ethical Committee (IAEC). The animal experiments were carried out as per Committee

for the Purpose of Control and Supervision on Experiments on Animals (CPSCSEA) guidelines and after IAEC approval.

Chemicals:

The chemicals Diazepam, Amitryptaline, L-dopa, and Chlorpromazine which were used for the present study were procured from Sd-Fine Chemicals Mumbai, Sigma Aldrich USA, Loba chemie Mumbai.

Test drug (4-hydroxy-3-[2-(3-nitrophenyl)-2, 3-dihydro-1,5-benzothiazepin-4-yl]-2h-chromen-2-one) was gifted by Prof. Anamik Shah, Department of chemistry, Saurashtra University, Rajkot-360005 Gujarat (INDIA).

EXPERIMENTAL DESIGN

Experiment 1: The rats were divided in to three groups and each group had six animals treated with the respective drugs for 30 days. First group of rats were treated with DMSO and used as control. Second group of rats were administered with standard drug Amitryptaline (14mg/kg). Third group rats were administered with the test drug (5mg/kg). On 31st day of the experiment anti-anxiety activity was performed.

Experiment2:

The rats were divided in to three groups and each group had six animals treated with the respective drugs for 30 days. First group of rats were treated with DMSO and used as control. Second group of rats were administered with standard drug Diazepam (4mg/kg). Third group rats were administered with the test drug (5mg/kg). On 31st day of the experiment anti-depressant activity was performed.

Experiment3:

The rats were divided in to three groups and each group had six animals. First group of rats were treated with chlorpromazine (3mg/kg, i.p.) and used as control. Second group of rats were administered with Chlorpromazine (5mg/kg, i.p.) and after 30 minutes standard drug L-DOPA (150 mg/kg, o. p.) was administered. Third group of rats were administered with Chlorpromazine (5mg/kg, i .p.) and after 30 minutes test drug (5 mg/kg, o. p.) was administered.

Acute toxicity study of 1, 5-benzothiazepine synthetic formulation (As per OECD guide lines number: 423) [6].

Female Wistar rats (weight: 180-220g) were taken for the study and kept for overnight fasting. Next day, body weight was taken and synthetic drug was administered orally at a dose of 2000mg/kg in DMSO (OECD; 1991). The animals were observed twice daily for 14 days and body weight was taken. The same experiment was repeated once again on 3 rats (preferably female) as there was no observable clinical toxicity for the animals on the phase 1 study. Based on these observations 5 mg/kg dose of synthetic drug was selected.

ASSESSMENT OF ANTI-ANXIETY ACTIVITY

Elevated Plus Maze [7]:

The apparatus similar to that described by Gopala Krishna et al [7] was used. It consist of a plus shaped maze elevated 45 cm above ground level. It has two open (10 x50 cm) and two closed (10 x 50 x 40 cm) arms. The test rat was placed in the central square area (10x10cm) of the plus maze and time spent in open arm during a 10 min observation period was noted. Data for vehicle, diazepam and test drug treated groups were compared.

ASSESSMENT OF ANTIDEPRESSANT ACTIVITY

Forced swim test (FST) [8, 9]:

Rats were individually forced to swim in an open cylindrical container (diameter 30 cm, height 60 cm), containing water of 45 cm depth at 25 ± 1 ºC. The test employed is essentially similar to earlier procedure. The water level has been increased (45 cm) in order to increase the sensitivity of the test. The rats lack a sense of the water's depth and their tails do not touch the bottom of the cylinder. Animals were exposed to a pre-test for 10 min, 3 day prior to the swim test. Each animal was considered immobile when it ceased to struggle and swim and remained floating in the water, only moving to keep its head above water. After the test, the animals were removed from the water, dried and placed in the cages. After 30 min of pre-test, the same animals were treated by drugs and the same procedure was repeated.

ASSESSMENT OF ANTI-CATALEPTIC ACTIVITY

Catatonia [9, 10]:

The major clinical symptom of Parkinson's disease includes difficulty to move and change the posture (akinesia and rigidity) and tremors. So by this parameter we could observe the severity of catatonia (Zazpe et al., 2006) as fallowed; Stage I- Rat moves normally when placed on table= (Score- 0). Stage II- Rat moves only when touched/pushed= (Score- 0.5). Stage III- Rat placed on the table with front paws set at least on a 3 cm high block fails to correct the posture in 10 sec= (Score- 0.5 for each paw total score-1). Stage IV- Rat fails to remove when front paws are placed alternately on 9 cm block= (score-1 for each paw total Score- 2). Thus for a single rat maximum possible score would be 3.5 revealing total catatonia.

Results

The effect of the 1, 5-benzothiazapine derivatives on anti-anxiety activity was given in **Table -1.** The treatment with Diazepam (4 mg/kg) and Test drug (5mg/kg) showed significant increase in the number of open arm entries 3.35, ($P<0.01$) and 3.0 ($P<0.01$) respectively and also spent more time in open arm, when compared with the control group (DMSO treated). Animal received only DMSO (control) had shown much decrease in the number of open arm entries 2.355($P<0.01$) and also spend less time in open arm.

The effect of the 1, 5-benzothiazapine derivatives on anti-depressant activity was given in **Table -2.** The behavioral score of immobility in control, standard drug and test drug treated groups were compared. Single dose administration of test drug showed significant decrease in immobility time as compared to control ($P<0.01$) and the effect was quantitatively comparable to the standard anti-depressant drug, amitryptaline also. The mobility time was significantly increased in standard and test drug when compared to solvent control (DMSO) group.

The effect of the 1, 5-benzothiazapine derivatives on anti-cataleptic activity was given in **Table -3.** The treatment with 1, 5–benzothiazepines showed significant anti-cataleptic actions; the chlorpromazine –induced catalepsy scores were significantly ($p<0.01$) reduced in the test drug treated animals, when compared to the rats injected with chlorpromazine alone. The effects

which were seen within 120 minutes and lasted for more than 180 minutes, compared well with those produced by L-Dopa (150 mg/kg i.p -30 min).

Table: 1

Evaluation of anti-anxiety activity of 1, 5-Benzothiazepine derivative by elevated plus maze in rats.

Sr. No	Treatment	Dose	Number of entries		Time spent (sec)	
			Open arm	Closed arm	Open arm	Closed arm
1.	Control	1 ml/kg	2.355± 0.022	2.651± 0.0289	91.83 ± 1.815	508.166±1.815
2.	Diazepam	4 mg/kg	3.353±0.0357**	2.0583±0.073**	266.83±4.40**	333.166±4.40**
3.	Test Drug	5 mg/kg	3.0 ± 0.0465**	1.966±0.044**	175.33±4.773**	424.66±4.773**

The values are mean ± S.E.M, **P<0.01 significantly different from their respective vehicle treated control groups. (ANOVA followed by Dunnett's 't' test).

Table: 2

Evaluation of anti-depressant activity of 1, 5-Benzothiazepine derivative by forced swim test (FST) in rats.

Sr. No	Treatment	Dose	Mobility time (sec)	Immobility time (sec)
1.	Solvent control	1 ml/kg	270.66 ± 1.745	29.33 ± 1.745
2.	Amitriptyline	14 mg/kg	292.166 ± 0.6009**	7.833 ± 0.6009**
3.	Test drug	5 mg/kg	289.166 ± 2.574**	10.833 ± 2.574**

The values are mean ± S.E.M, **P<0.01 significantly different from their respective vehicle treated control groups. (ANOVA followed by Dunnett's 't' test).

Table: 3

Assessment of anti-cataleptic activity of 1, 5-Benzothiazepine derivative by using rats.

Sr. No	**Treatment**	**Dose**	**Catalepsy score**
1.	Chlorpromazine	5 mg/kg	1.333 ± 0.1667
2.	L-DOPA+ Chlorpromazine	150 mg/kg	0.333 ± 0.1667**
3.	Test-drug+ Chlorpromazine	5 mg/kg	0.4166 ± 0.1537**

The values are mean ± S.E.M, **P<0.01 significantly different from their respective vehicle treated control groups. (ANOVA followed by Dunnett's't' test).

Discussion

Mood disorders are the most common problem among the world population. In elevated plus maze study the number of entries into open arms and closed arms, time spend in open arms and closed arms were taken as a measure of anxiety. Diazepam the known anxiolytic showed good anxiolytic activity and these results were compared with the test drug and also showed more over equipotent effect as that of diazepam when compared with the control animals.

Though animal models have their limitation in simulating aberrations of the human mind, the identification of anti-anxiety, anti-depressant and anti-cataleptic activity was being first time reporting in this synthesized compound. The effects compared well with standard drugs, such as diazepam amitriptyline and L-DOPA in various animal models. These drugs, despite being effective and fairly safe, are known to have some undesirable features eg. Sedative, amnesic, ataxia, tolerance and physical dependence.

The anti-depressant activity was proved by forced swim test. The administration of test drugs significantly increased the mobility time and it decreases the immobility time. The anti-cataleptic activity was pronounced with the test drug as that of L-DOPA treatment groups. Various clinical and experimental reports also indicated that tricyclic anti-depressant drugs were useful in the

treatment of acute and chronic pain conditions via the participation of endogenous opioid system and partly by further activating nor adrenergic and serotonergic pathway.

Animal models of depression have certain limitation as they are based on ability to support animal behavior in stressful situation that ordinarily leads to decreased responsiveness (learned helplessness). The possible actions involved in these effects may be involved through dopaminergic and serotonergic actions. The anti-cataleptic actions of this compound may be due to anti-cholinergic or it may have a dopamine facilitating actions in the CNS. The strength of 1,5-benzothiazipine nucleus is evident from the clinical uses such as cardiovascular and CNS.

Conclusion

The present study conducted with 1, 5-benzothiazipine for CNS activities was successful. It showed a marked anti-anxiety, anti-depressant and anti-cataleptic actions. For understanding the mode of action of these compounds, it needs a detailed pharmacological and biochemical studies. If it proven as a good experimental drug for CNS ailments, it may lead to a potent clinical therapeutic agent in the future.

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