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Potentiation of pressor effect of tyramine by newly synthesized compound 2[(n- benzylacetamido) mercapto] benzimidazole (vs 25), a putative inhibitor of monoamine oxidase–A enzyme in rats

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ABSTRACT

A series of compounds were synthesized with potential of antidepressant activity. Preliminary research work indicated that monoamine oxidase inhibitor may one of the mechanism of action. Interaction of monoamine oxidase inhibitors with tyramine containing food material is known. Thus aim of the present investigation is to evaluate the pressor effect of tyramine in rats pretreated with synthesized compound (VS 25) and moclobemide. The dose response relationship to tyramine was determined by injecting tyramine i.v. in anaesthetized rats. The study protocol was divided into two parts 1 day treatment model and 14 day treatment model. In one day treatment model, saline control group showed no significant change in SBP,DBP, MABP and heart rate. In cmc control group 1st injection of tyramine showed rise in blood pressure 195.60±8.40 from initial reading of 119.82± 5.62(baseline) before tyramine and subsequent injection decreased blood pressure. Moclobemide 50 mg/kg and VS 25 (30 and 60mg/kg) significantly p<0.01 increased SBP, DBP and MABP. The 14 day treatment model showed significant potentiation of pressor effect of tyramine as compared to 1 day drug treatment model p<0.001. The heart rate decreased in both the model and on 14 day there tachycardia was observed compared to moclobemide. On comparison of 1 day and 14 day treatment model VS 25 (30 mg/kg), VS 25 (60 mg/kg) and moclobemide (50 mg/kg) showed similar rise in SBP, DBP and MABP. Both the drugs failed to prevent tyramine tachyphylaxis. While VS25 (30 mg/kg) showed significant (p<0.001) potentiation of pressor effect of tyramine in 14 day treatment model. Heart rate increased in both VS 25 (30 and 60mg/kg) treated rats. After injection of tyramine (2mg/kg) indicating tachycardia compared to moclobemide and cmc control. Subsequent doses showed mild tachycardia. It is concluded that cardiovascular activity of VS 25 (30 mg/kg) may be due to MAO A inhibition as the potentiation of tyramine pressor response by VS 25 was similar to that of moclobemide.

Key words: Depression, MAO inhibitor, tyramine, Pressor effect, VS 25.

INTRODUCTION

Monoamine oxidase (MAO) the principle enzyme responsible for the regulation and the catabolism of monoamines has been found to be involved in the depression is located in the outer mitochondrial membrane of many tissues. Inhibition of this enzyme on administration of MAO inhibitor (MAOI) leads to increase the concentration of monoamine in central nervous system (CNS)[1]. Tyramine is an indirectly acting sympathomimetic amine which is actively transported to sympathetic nerve ending through the neuronal reuptake mechanism [2]. It acts by releasing

neurotransmitter norepinephrine (NE), from adrenergic axonal terminal, which in turn increase in systolic blood pressure[3].

The major reason for the restricted clinical use of MAOI is the occurrence of hypertensive crises after ingestion of tyramine rich food and beverages such as beer, red wine, aged cheeses, broad beans in patients on MAOI, yeast extract and soy sauce, banana peels, meat extract and buttermilk[4] .

The factors contribute to a potentiation of pressor effect of tyramine are-
a) Normally tyramine is metabolized in liver by MAO enzymes. MAOI by inhibiting detoxification lead to tyramine accumulation which result in release of norepinephrine from binding sites causing a marked rise in blood pressure. b) Metabolic inactivation of tyramine taken up into sympathetic nerve ending and c) prevention of displaced norepinephrine deamination [3,5] .

In recent years, moclobemide, a reversible inhibitor of monoamine oxidase-A (RIMA), has been reported to be safer than older MAOIs, particularly in terms of hypertensive crisis and it is because of its reversibility that inhibitors could be displaced from the active site of MAO by high concentration of substrate, e.g., tyramine [6]. These compounds (RIMA) are reported to have anti-parkinsonian activity and neuroprotective effects in a cerebral ischemia model, and these effects appear to be independent of inhibition of MAO-A [7]. This RIMA has also been reported to facilitate differentiation of stem cells into functional neurons [8] and to reduce anoxia and glutamate-induced neuronal damage in cerebral cortex cultures [9].

Considering the history of monoamine oxidase inhibitor safety is the important factor from clinical point of view. In order to develop safe and effective monoamine oxidase inhibitor. A series of compounds were synthesized out of which 2[(N-benzylacetamido) mercapto] benzimidazole] labeled as VS-25 was selected based on QSAR and drug docking system[10].

The objective of the present investigation was to evaluate the pressor effect of tyramine in rats pretreated with synthesized compound (VS 25) and moclobemide.

MATERIALS AND METHODS

2.1. Selection of doses

The selection of doses of test compound VS 25 (30 mg/kg and 60 mg/kg) was based on the equivalent dose of moclobemide 50 mg/kg.

2.2. Drugs and chemicals

Tyramine (P-(2 aminoethyl)-phenol-hydrochloride) was obtained from SRL Research laboratory private Ltd., Mumbai, India. Moclobemide® (Trima 100) Intas pharmaceuticals Ltd., Mumbai., India and, Sodium carboxy methyl cellulose (CMC) (Research- Lab, India) were purchased from local vendors.

The test compound VS-25 was synthesized by Dr. Suhas Shelke, department of pharmaceutical chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, India and supplied to us for evaluation of pharmacological activity.

2.3. Preparation of drug solution:-

a) VS 25 and moclobemide were suspended in 1% Sodium carboxy methyl cellulose (CMC) solution. The route of administration was oral.
b) Tyramine was dissolved in normal saline (1 mg/kg.). The route of administration was intravenous.

2.4. Experimental animals:-

Male Wistar rats (200–250 g) and Swiss albino mice (18-22gm) were procured from National Toxicology Centre, Pune, India and housed in animal house in groups of six animals in polypropylene cages. The animals were housed at 25±2 °C, relative humidity of 45% to 55% and under standard environmental conditions of 12 h light 12 h dark cycle. All the animals were acclimatized for 10 days to the animal house conditions prior to the start of experimental protocol. The animals had free access to food (Amrut laboratory animal feed, Sangali, MS, India) and water *ad libitum*.

The research protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted as per the directions of the committee for the purpose of control and supervision of experiment on animals (No: CPCSEA31/14). All experiments were carried out between 12:00-16:00 hours

2.5. Acute Toxicity Study

Acute toxicity study was carried out as per OECD guidelines- 425. Five female Swiss albino mice were used for studies which were fasted overnight, providing free access to water. The test compound was administered orally at one dose level of 2000 mg/kg body weight and animals were observed continuously for the first 4 h and then periodically up to 24 h for toxicity and mortality.

2.6. Experimental design and protocol

Male wistar rats (200-250g) were divided into following five groups of six rats each.

Groups	Treatment	Dose	Tyramine doses
Group 1	CMC control	20mg/kg(orally)	1mg/kg & 2mg/kg
Group 2	Moclobemide	50mg/kg (orally)	1mg/kg & 2mg/kg
Group 3	VS- 25	30mg/kg (orally)	1mg/kg & 2mg/kg
Group 4	VS- 25	60 mg/kg (orally)	1mg/kg & 2mg/kg

The study protocol was divided into two parts 1 day treatment model and 14 day treatment model. In one day treatment model, moclobemide and VS 25 was administered to rats orally,1 hour prior to urethane anaesthesia. In 14 day treatment model, rats received moclobemide and VS 25 once a day, orally for 14 day.

2.6.1. Anaesthesia and Surgical Preparation for recording haemodynamic parameter:-

Male Wistar rats weighing between 200- 250 g were anaesthetized with urethane (1.25 g/kg, i.p.). The systemic blood pressure was recorded from the right common carotid artery by arterial canulation for the measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MABP). Simultaneously for the measurement of electrocardiogram (ECG) and heart rate, the (ECG) leads were placed on the right foreleg (negative electrode), left foreleg (positive electrode) and right hind leg (neutral electrode). The haemodynamic parameters were recorded by eight channel recorder Power lab having LABCHART-6 pro software by means of an acquisition data system (AD Instruments Pty Ltd with LABCHART 7 pro software, Australia). The left femoral vein of the rats was cannulated for the administration of tyramine.

2.6.2. Measurement of pressor response of tyramine after i.v. administration

The dose response relationship of tyramine was determined by injecting tyramine(i.v.) through the left femoral vein. Tyramine was injected at increasing doses 1 mg/kg- 2mg/kg respectively. The subsequent higher dose was injected after SBP had returned to baseline. The doses of tyramine were gradually increased until a SBP response of ≥30mm of Hg occurred. According to Korn et al (1996) this dose was considered to be the smallest "effective dose" or smallest pressor dose [3].

2.7. Statistical Analysis :-Values were expressed as mean ± SEM and The data were analyzed by two way ANOVA followed by Bonferroni's post hoc test. The statistical analyses were performed using Graph Pad Prism 5 software (San Diego, CA). Data was considered statistical significant at $P<0.05$.

RESULTS

3.1. Effect of tyramine on SBP, DBP and MABP of test compound treated animals (1 day)

In saline control group no significant change in systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and heart rate did not alter after repeated injection of saline (i.v.) at increasing doses 0.4ml to 0.8 ml respectively. (Fig-1)

In cmc control group 1st injection of tyramine showed rise in SBP, DBP and MABP 195.60±8.40 mm of Hg , 172.68 ± 7.53 mm of Hg and 172.64 ± 3.67mm of Hg from initial reading of 119.82± 5.62 mm of Hg , 106.60 ±5.95 mm of Hg and 119.76 ± 8.89 mm of Hg (baseline) before tyramine. The SBP, DBP and MABP increase by 75.78 mm of Hg , 66.08 mm of Hg and 52.88 mm of Hg was significant ($p<0.001$)compared to control.

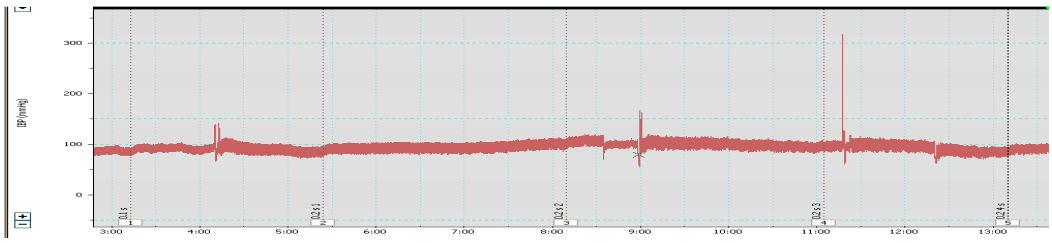


Fig 1 :- Saline control group was treated with cmc and saline (vehicle for tyramine)

After higher dose of tyramine injection (2mg/kg) the SBP, DBP and MABP readings were 204.70±13.19 mm of Hg , 181.38± 4.92 mm of Hg and 211.82±7.71 mm of Hg respectively, therefore this dose of 2mg/kg was selected for repeated administration. Subsequent doses of tyramine (2mg/kg) failed to raise the blood pressure to be extend shown by the first dose of 2mg/kg indicating tachyphylaxis.

In moclobemide (50mg/kg) treated rats 1st dose of 1mg/kg tyramine showed non significant increase in the SBP, DBP and MABP compared to that of cmc control group. The first dose of 2mg/kg showed significant ($p<0.05$) increase in SBP and significant ($p<0.01$) increase in DBP and MABP, however the subsequent doses showed non significant increase in the blood pressure. The results confirmed presence of tachyphylaxis with subsequent doses of tyramine. The rise in blood pressure by the 1st dose of 2 mg/kg tyramine indicated that in moclobemide treated animals there is a potentiation of the pressure response of tyramine.

In rats treated with VS 25 (30mg/kg) the lower and higher doses of tyramine i.e. 1mg/kg and 2mg/kg showed increase in SBP, DBP and MABP ($p<0.01$) similar to that of moclobemide. Subsequent doses of tyramine (2mg/kg) showed significant decrease in tyramine responses compared to that of first dose (2 mg/kg) indicating failure of inhibition of tyramine induced tachyphylaxis.

VS25 (60mg/kg) treated rats also showed potentiation of tyramine effect by first dose of 2 mg/kg and failure of inhibition of tyramine tachyphylaxis on subsequent doses. The result thus indicated that VS25 and moclobemide showed close proximity in potentiation of tyramine (2mg/kg) induced pressor response by 1st dose and both the drugs failed to prevent tyramine tachyphylaxis (Table- 1, 2,3and Fig 2).

Table 1.Effect of tyramine on systolic blood pressure (SBP mm of Hg) of test compound treated animals (1 day treatment)

Groups/ doses	Baseline	1mg/kg	2mg/kg	2mg/kg	2mg/kg	2mg/kg
CMC control	119.82±5.62	195.60±8.4	204.70±13.19	184.70±4.5	171.50±8.73	160.73±9.35
Moclobemide(50 mg/kg)	115.24± 2.17	225.08 ±9.48^{ns}	242.30 ±4.81*	217.46 ± 6.85*	204.72±7.67*	199.20±3.77*
VS 25 (30 mg/kg)	122.35±10.98	238.41±7.44**	250.52±12.87**	231.75 ± 8.66***	217.27±6.98 **	202.20±7.91**
VS 25(60 mg/kg)	123.09± 8.29	235.66± 9.91**	243.86± 5.99**	226.47± 8.25**	205.51± 5.11*	191.44±7.06^{ns}

*Values were expressed as mean ±SEM and statistical ly analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying Two Way ANOVA followed by Bonferroni's post hoc test,*** p< 0.001,**p<0.01,* p< 0.5 was considered to be significant as compare to cmc control.*

Table 2. Effect of tyramine on diastolic blood pressure (DBP mm of Hg) of test compound treated animals (1 day treatment)

Groups/ doses	Baseline	1mg/kg	2mg/kg	2mg/kg	2mg/kg	2mg/kg
CMC control	106.60±5.95	172.68±7.53	181.38±4.92	143.31 ±3.00	131.84 ±7.05	118.44 ±4.31
Moclobemide (50 mg/kg)	97.89 ±1.75	189.51 ±3.33^{ns}	228.67± 11.23**	181.49 ±12.81*	172.97 ±4.08**	168.29± 5.18***
VS 25 (30 mg/kg)	108.56±1.62	212.37±12.70**	227.29± 11.39**	189.09 ±6.32**	178.18 ±12.37**	169.51 ±11.24**
VS 25(60 mg/kg)	121.77±3.92	193.52 ±7.47^{ns}	221.01 ±18.94**	174.01 ±13.66^{ns}	163.06 ±12.05^{ns}	149.82 ±16.14^{ns}

*Values were expressed as mean ±SEM and statistical ly analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying Two Way ANOVA followed by Bonferroni's post hoc test,*** p< 0.001,**p<0.01,* p< 0.05 was considered to be significant as compare to cmc control.*

3.2. Effect of tyramine on heart rate of test compound treated animals (1 day treatment).

The heart rate in cmc (vehicle) treated rats was 265.08±14.6 beats per minute (bpm) before tyramine administration. The heart rate after tyramine injection (1st dose mg/kg) decrease to 247.15±14.49 bpm. But the first dose of tyramine (2 mg/kg) increase the heart rate to 262.84±12.71 bpm which was still less than that compared to initial rate of 265.08±14.6 The subsequent doses of tyramine (2 mg/kg) showed decrease in heart rate.

Table 3. Effect of tyramine on mean arterial blood pressure (MABP mm of Hg) of test compound treated animals (1 day treatment).

Groups/ doses	Baseline	1mg/kg	2mg/kg	2mg/kg	2mg/kg	2mg/kg
CMC control	119.76± 8.89	172.64 ±3.67	211.82 ±7.71	184.95 ±8.44	153.80 ±7.83	128.76 ± 9.48
Moclobemide(50 mg/kg)	108.65± 7.40	219.16 ±11.49*	270.05 ± 15.76**	229.72 ± 12.33*	199.47 ± 6.44*	190.05 ±4.32**
VS 25 (30 mg/kg)	116.18 ±15.07	224.49 ± 8.80*	268.70 ± 3.44**	244.96 ±18.63**	206.33 ±15.21**	188.18 ±15.08**
VS 25(60 mg/kg)	115.52 ±15.17	215.50 ±13.38^{ns}	264.98 ±8.68**	207.72 ±9.84^{ns}	197.2 ± 15.75^{ns}	194.72 ±21.27***

*Values were expressed as mean ± SEM and statistical ly analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying Two Way ANOVA followed by Bonferroni's post hoc test,*** p< 0.001,**p<0.01,* p< 0.05 was considered to be significant as compare to cmc control.*

1st dose of 2mg/kg of tyramine in moclobemide treated rat showed decrease in heart rate 259.56±6.41 beats/min and subsequent doses also decreased the heart rate. In both VS 25 (30 mg/kg and 60 mg/kg) treated rats tyramine administration produced increase in heart rate 287.12±17.56 beats/min and 291.36±8.36 beats/min indicating tachycardia. (Table- 4).

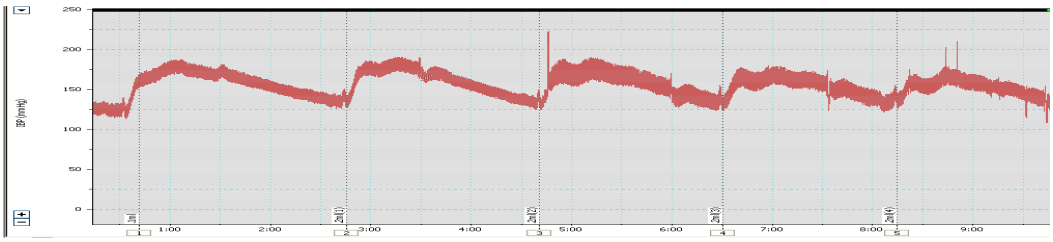
Table 4. Effect of tyramine on heart rate (baets/min) of test compound treated animals. (1 day treatment)

Groups/ doses	Baseline	1mg/kg	2mg/kg	2mg/kg	2mg/kg	2mg/kg
CMC control	265.08±14.6	247.15±14.49	262.84±12.71	249.63±20.25	243.13±16.95	238.79±16.57
Moclobemide(50 mg/kg)	263.3±8.22	253.92±11.08ns	259.56±6.41ns	245.07±9.48ns	237.85±13.01ns	234.15±13.29ns
VS 25 (30 mg/kg)	251.93±14.06	273.32±12.16ns	287.12±17.56ns	252.54±13.91ns	249.23±11.73ns	247.98±12.97ns
VS 25(60 mg/kg)	259.94±9.38	282.97±13.97ns	291.36±8.36ns	262.04±10.86ns	255.52±10.27ns	255.11±11.95ns

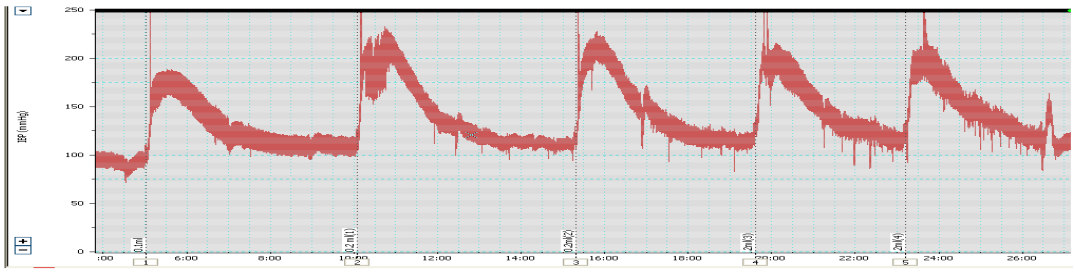
Values were expressed as mean ± SEM and statistical ly analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying Two Way ANOVA followed by Bonferroni's post hoc test, ns p> 0.05 was considered to be significant as compare to cmc control.

Blood pressure recording of 1 day treatment model

Fig 2:- Effect of tyramine (i.v) on blood pressure of rats treated with A-cmc control, B-Moclobemide, C- VS (30mg/kg) and D-VS 25 (60 mg/kg) for 1 day



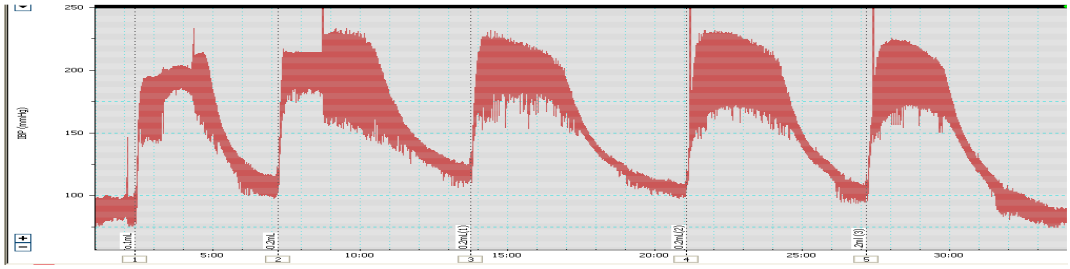
Group A:- was treated with cmc and tyramine (vehicle for test and standard drugs)



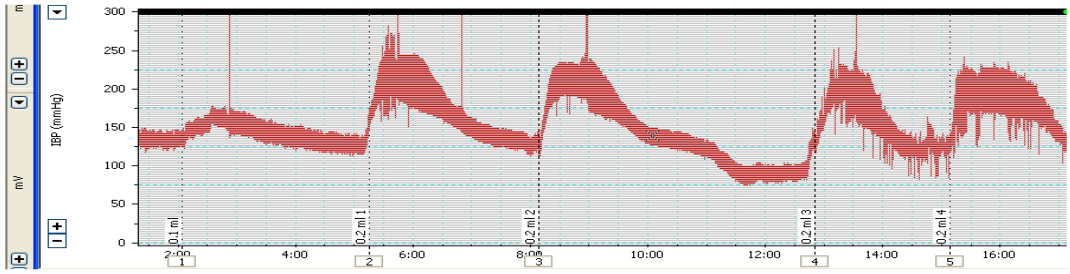
Group B :- was treated with moclobemide and tyramine (standard drugs)

3.3.Effect of tyramine on SBP, DBP and MABP of test compound treated animals (14 day)

In cmc control group 1st injection of tyramine showed rise in SBP 215.13±6.26 mm of Hg, DBP 147.83 ±17.44 mm of Hg and MABP 168.59 ± 19.52 mm of Hg from initial reading of 136.59±14.95mm of Hg, 126.79 ±14.49 mm of Hg and 134.08 ± 14.36mm of Hg(baseline) before tyramine. The SBP increase by 78.54 mm of Hg was significant (p<0.05) and the rise in DBP and MABP 21.04 mm of Hg and 34.51 mm of Hg was non-significant (p>0.05) as compared to control.



Group C :- was treated with VS 25 (30mg/kg) and tyramine (test drugs at lower doses)



Group D :- was treated with VS 25 (60mg/kg) and tyramine (test drugs at higher doses)

After first higher dose of (2 mg/kg) increases blood pressure was observed but subsequent doses failed to raise the blood pressure to be extend shown by the first injection of tyramine indicating tachyphylaxis.

In moclobemide 50mg/kg treated rats 1st dose of tyramine (1mg/kg) showed non significant increase in the SBP, DBP and MABP compared to that of cmc control group. This rise in SBP, DBP and MABP was less as compared to VS 25 (30 mg/kg) and greater as that of test drug VS 25 (60 mg/kg), representing more potentiation of pressor effect by VS 25 (30 mg/kg) compared to moclobemide and higher dose of VS 25 (60 mg/kg). The results confirmed presence of tachyphylaxis with subsequent doses of tyramine. The rise in SBP,DBP and MABP indicates that in moclobemide treated animals there is a potentiation of the pressure response of tyramine.

In rats treated with VS 25 (30mg/kg) 1st injection of 2mg/kg showed significant increase in the SBP, DBP and MABP ($p<0.001$) than that of moclobemide. Subsequent doses of tyramine 2mg/kg showed significant decrease in tyramine responses compared to that of 1st dose of 2 mg/kg indicating failure of inhibition of tyramine induced tachyphylaxis.

VS25 (60mg/kg) treated rats showed non significant increase in the SBP, DBP and MABP as compared to cmc control group, potentiation of tyramine effect and non inhibition of tyramine tachyphylaxis was also observed similar as that of VS 25 (30mg/kg) but to a lesser extend. The result thus indicated that VS25 (30mg/kg) showed more potentiation as compared to moclobemide and VS 25 (60mg/kg), both the doses of test drug showed potentiation of tyramine induced pressure response(Table- 5,6,7and Fig-3).

Table 5. Effect of tyramine on systolic blood pressure (SBP mm of Hg) of test compound treated animals (14 day treatment)

Groups/ doses	Baseline	1mg/kg	2mg/kg	2mg/kg	2mg/kg	2mg/kg
CMC control	136.59 ±14.95	215.13 ± 6.26	205.03± 8.67	175.11± 21.11	157.73± 19.14	145.98± 29.14
Moclobemide(50mg/kg)	157.12± 18.73	268.63± 13.78ns	301.83± 21***	250.52± 15.88*	240.57± 13.06**	215.85± 14.61*
VS 25 (30 mg/kg)	180.71± 47.55	302.00± 38.03**	352.34± 7.87***	297.3± 12.55***	272.8± 16.49***	247.82±11.9***
VS 25(60 mg/kg)	138.65± 9.92	207.47± 17.62ns	264.56± 8.54ns	213.06± 14.25ns	196.12± 13.54ns	165.44± 14.74ns

*Values were expressed as mean ±SEM and statistical ly analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying Two Way ANOVA followed by Bonferroni's post hoc test,*** p< 0.001,**p<0.01,* p< 0.05 was considered to be significant as compare to cmc control.*

Table 6. Effect of tyramine on diastolic blood pressure (DBP mm of Hg) of test compound treated animals (14 day treatment)

Groups/ doses	Baseline	1mg/kg	2mg/kg	2mg/kg	2mg/kg	2mg/kg
CMC control	126.79 ± 14.49	147.83 ± 17.44	139.57 ± 9.88	150.07 ± 13.64	139.95 ± 10.9	127.49 ± 8.73
Moclobemide(50mg/kg)	144.56 ± 19.24	228.41 ± 15.13^{ns}	262.83 ± 20.01**	261.48 ± 15.57**	231.06 ± 13.57*	218.48 ±14.06*
VS 25 (30 mg/kg)	151.8 ± 48.9	234.95 ± 45.58^{ns}	272.07 ± 42.75***	276.18 ± 46.21**	232.74 ± 49.99*	234.37 ± 49.51*
VS 25(60 mg/kg)	124.26 ± 8.85	182.95 ± 9.07^{ns}	234.37 ± 10.72*	179.61 ± 9.66^{ns}	190.35 ± 6.67^{ns}	187.96 ± 5.37^{ns}

*Values were expressed as mean ±SEM and statistical ly analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying Two Way ANOVA followed by Bonferroni's post hoc test,**p<0.01,* p< 0.05 was considered to be significant as compare to cmc control.*

Table 7. Effect of tyramine on mean arterial blood pressure (MABP mm of Hg) of test compound treated animals (14 day treatment)

Groups/ doses	Baseline	1mg/kg	2mg/kg	2mg/kg	2mg/kg	2mg/kg
CMC control	134.08 ± 14.36	168.59 ± 19.52	143.17 ± 21.84	155.99 ± 17.29	145.72 ± 12.68	141.9 ± 12.5
Moclobemide (50mg/kg)	153.92 ± 18.6	243.38 ± 15.72^{ns}	270 ± 23.79**	259.03 ± 18.3*	245.86 ± 18.68*	239.46 ±21.17*
VS 25 (30 mg/kg)	165.14 ± 48.95	249.07 ± 43.89^{ns}	290.01±44.48***	285.15±48.15**	273.57 ±49.33**	265.77 ±44.81**
VS 25(60 mg/kg)	132.05 ± 9.34	197.61 ± 14.26^{ns}	250.58 ± 14.73*	195.26 ± 10.16^{ns}	204.44 ± 8.94^{ns}	201.61 ± 7.75^{ns}

*Values were expressed as mean ±SEM and statistical ly analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying Two Way ANOVA followed by Bonferroni's post hoc test,*** p< 0.001,**p<0.01,* p< 0.05 was considered to be significant as compare to cmc control.*

3.4. Effect of tyramine on heart rate of test compound treated animals (14 day treatment).

The heart rate in cmc (vehicle) treated rats was 266.13±13.04 beats per minute (bpm) before tyramine administration. The heart rate after tyramine injection (1st dose mg/kg) decrease to 265.17±20.22 bpm. But the first dose of tyramine (2 mg/kg) increases the heart rate to 297.23±14.99 bpm which was greater as compared to initial rate of 266.13±13.04 beats per minute. The subsequent doses of tyramine (2 mg/kg) showed decrease in heart rate. 1st dose of 2mg/kg of tyramine in moclobemide treated rat showed increase in heart rate 308.7±20.64 beats/min and subsequent doses showed decreased in the heart rate. In both VS 25 (30 mg/kg and 60 mg/kg) treated rats tyramine administration produced increase in heart rate 361.57±18.12^{ns} beats/min and 361.38±10.58 beats/min than that of moclobemide and cmc treated groups indicating tachycardia(Table-8).

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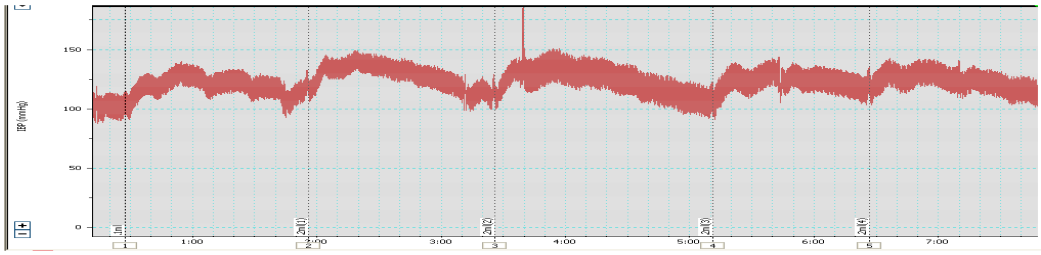
Table 8. Effect of tyramine on heart rate (baets/min) of test compound treated animals (14 day treatment)

Groups/ doses	Baseline	1mg/kg	2mg/kg	2mg/kg	2mg/kg	2mg/kg
CMC control	266.13±13.04	265.17±20.22	297.23±14.99	289.79±25.33	260.44±23.31	235.96±19.52
Moclobemide(50mg/kg)	280.29±12.15	268.27±22.72^{ns}	308.7±20.64^{ns}	264.00±27.00^{ns}	249.57±24.39^{ns}	228±17.5^{ns}
VS 25 (30 mg/kg)	281.01±14.34	263.04±12.9^{ns}	361.57±18.12^{ns}	339.63±12.38^{ns}	269.24±19.74^{ns}	267.32±7.36^{ns}
VS 25(60 mg/kg)	276.01±11.84	274.47±16.99^{ns}	361.38±10.58^{ns}	331.29±9.47^{ns}	260.65±12.19^{ns}	257.26±11.67^{ns}

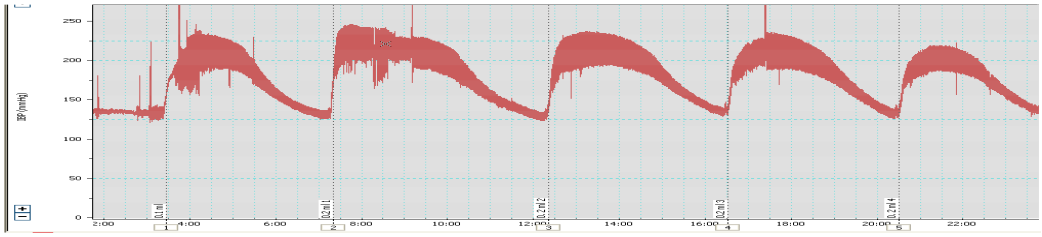
Values were expressed as mean ±SEM and statistical ly analysis was carried out using Graph Pad 5.0 software (Graph Pad, San Diego, USA) by applying Two Way ANOVA followed by Bonferroni's post hoc test, ns p > 0.05 was considered to be statistically non significant.

Power lab tracing of 14 day treatment model

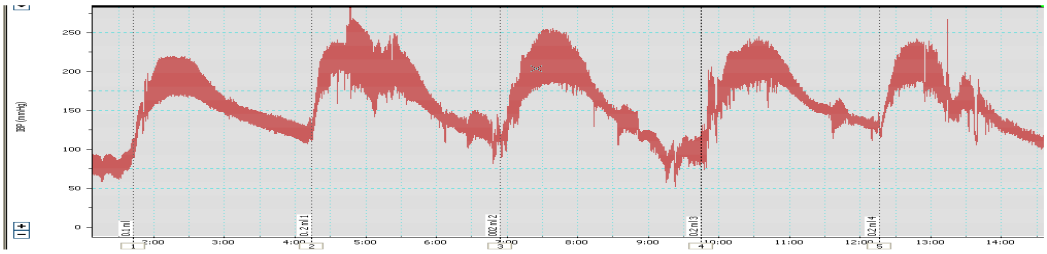
Fig 3:- Effect of tyramine (i.v) on blood pressure of rats treated with A-cmc control, B-Moclobemide, C- VS (30mg/kg) and D-VS 25 (60 mg/kg) for 14 day



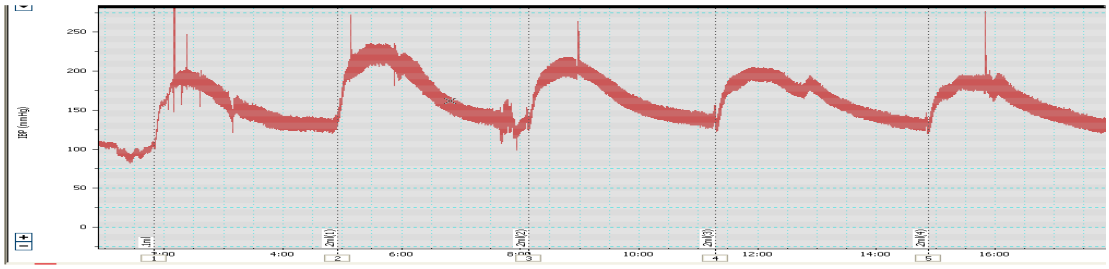
Group A :- was treated with cmc and tyramine (vehicle for test and standard drugs)



Group B :- was treated with moclobemide and tyramine (standard drugs)



Group C :- was treated with VS 25 (30mg/kg) and tyramine (test drugs at lower doses)



Group D :- was treated with VS 25 (60mg/kg) and tyramine (test drugs at higher doses)

DISCUSSION

Historically monoamine oxidase inhibitors, were used as first line antidepressants introduced in late 1950s, continue to have a niche in the treatment of psychiatric and neurological disorders. MAO inhibitor are reported to have clinical effectiveness in controlling anxiety disorder such as panic disorder and Parkinson's, social phobia and in treatment of atypical depression [11,12,13] . In addition, some patients with typical endogenous depression who do not respond well to tricyclic antidepressants may respond very favorably to MAOIs [14]. Despite those benefits the first effective antidepressant drug Iproniazid was introduced in 1951. Iproniazid is a hydrazine derivative, was reported to have mood-elevating effects in patients with tuberculosis, but owing to hepatotoxicity was abandoned because of the adverse effect: hepatotoxicity [15,16]. Other limitation of Iproniazid is their ability to potentiate cardiovascular effect of tyramine resulting in a life threatening hypertensive crises so called cheese reaction[6,17,18].

Hypertensive crises is the major adverse effect which is most commonly related to irreversible monoamine oxidase inhibitor following the oral administration of tyramine containing food in animals and in human being which restrict the clinical use of monoamine oxidase inhibitor MAOI[19,20,5,21] and intravenous tyramine[22,23,24].

More number of studies have been reported which clarify the mechanism of action of tyramine. Cohn in 1965 showed that the i.v. infusion of tyramine increased the MABP, decreased heart rate and no change in cardiac output. He has reported that tyramine act by increasing peripheral vascular resistance in man[25]. Korn et al.,(1986) showed that tyramine does not produce significant changes in pressor effect on 1 hour prior administration of moclobemide in contrast 1 week administration produces severe cheese reaction in healthy volunteers[26]. Similar findings were observed in the present study where the pressor effect is induced by intravenous administration of tyramine in Wistar rats. In one day drug treatment model 1 hour after moclobemide and VS 25 (30 and 60 mg/kg) treated

tyramine injected intravenously increased pressor effect. Significant potentiations of pressor effect of tyramine were observed in VS 25(30 gm/kg). The 2 week of administration of VS 25 treatment resulted in potentiation of pressor effect of tyramine. Colombo et al.,(1988) demonstrated that prior treatment with moclobemide resulted in tyramine caused a temporary dose dependent increase in systolic and diastolic blood pressure, whilst the heart rate remained unaffected in man[2] . Korn et al., (1988) studied the effect of moclobemide on absorption and pressor effect of tyramine and stated that on i.v. injection of tyramine 1 hour after moclobemide showed significant increase in SBP compared to control[27]. Burkard et al., (1989) demonstrated the neuroprotective profile of the moclobemide with minimal tyramine potentiating activity in freely moving rats and he stated that there is increase in MABP in moclobemide treated group compared to predrug treated rats[5]. Gal et al., (2010) reported that increasing dose of tyramine increases the MABP more than 30mmof Hg in Sprage Dawley rats[28].

In the present study VS 25 (30mg/kg and 60 mg/kg) treated animals showed significant increase in SBP, DBP and MABP. Heart rate remained unaffected in moclobemide control group while VS 25(30 and 60 mg/kg) showed increase in heart rate which represented mild tachycardia.

According to Cantarini et al., (2004) and Fankhauser et al., (1994) target rise of 30 mm of Hg in SBP was considered to be sufficient size to be attributable to tyramine rather than natural variability and also reported by increasing dose of tyramine the SBP increases[20,22]. Our results showed that there is significant rise in the blood pressure in tyramine control group as compared to vehicle treated group. In our study all rats showed tyramine sensitivity as the SBP increased by > 30mm of Hg.

In one day treatment model systolic blood pressure, diastolic blood pressure, mean arterial blood pressure was significantly increase after 1st injection of 2mg/kg of tyramine (i.v.) and on administration of same dose of tyramine showed decrease in SBP representing tachyphylaxis.

1 mg/kg dose of tyramine is sufficient to increase the systolic blood pressure by 30 mm of Hg thus in the present study all the rats showed tyramine sensitivity in both the model. Maximum increase in systolic blood pressure was observed approximately 5-6 minute after tyramine administration

CONCLUSION

On comparision of 1 day and 14 day treatment model VS 25 (30 and 60 mg/kg), newly synthesized test compound VS (30 mg/kg) and moclobemide (50 mg/kg) showed similar rise in SBP, DBP and MABP thus showed closed proximity in potentiation of tyramine induced pressor response on administration of 1st dose of tyramine (2 mg/kg). Observed after both the drugs failed to prevent tyramine tachyphylaxis. While VS 25 (30 mg/kg) showed significant ($p<0.001$) potentiation of pressor effect of tyramine in 14 day treatment model.

Heart rate were found to increased in both VS 25 (30 and 60mg/kg) on injecting tyramine (2mg/kg) indicating tachycardia compared to moclobemide and cmc control. Subsequent doses showed mild tachycardia

It is concluded that activity of VS 25, newly synthesized compound (30 mg/kg) may be due to MAO inhibition. The potentiation of tyramine pressor response by VS 25 are similar to that of moclobemide .The observed effect may be due to MAOI activity of VS 25.

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