CHAPTER 3

ANTIDEPRESSANT-LIKE EFFECT OF VENLAFAXINE IN BEHAVIORAL PARADIGMS OF DESPAIR: POSSIBLE MECHANISM OF ACTION

Part-1: Antidepressant activity of venlafaxine in mouse forced swim test: Involvement of L-arginine-nitric oxide-cyclic guanosine monophosphate pathway

3.1.1. INTRODUCTION

Venlafaxine, a novel antidepressant is an inhibitor of both serotonin and norepinephrine transporters (SERT and NET, respectively) (Redrobe et al., 1998). It exhibits six- to seven- fold selectivity for inhibition of serotonin reuptake as compared to norepinephrine reuptake in synaptosomes of rat brain and a 15- to 30- fold higher affinity for SERT binding sites as compared to those of NET (Gould et al., 2006). Venlafaxine has been shown to be superior in efficacy to selective serotonin reuptake inhibitors (SSRIs) in severe major depressive disorder, treatment-resistant depression, depressive symptom remission and obsessive compulsive disorder (Gutierrez et al., 2003).

L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) is an important signaling pathway that is reported to be involved in depression (Mantovani et al., 2003). Nitric oxide, a messenger molecule in the brain, synthesized from L-arginine by nitric oxide synthase (NOS), and has been implicated in neurotransmission, synaptic plasticity, learning, perception of pain, aggression and depression (Esplugues, 2002). Recent evidences have shown that the reduction of NO levels within the hippocampus can induce antidepressant-like effects, thus implicating endogenous hippocampal NO in the neurobiology of stress and depression (Joca and Guimares, 2006). Nitric oxide is also known to
modulate the levels of cyclic guanosine monophosphate (cGMP) which in turn known to produce depression like state in animals (Kaster et al., 2005b). Recent studies have shown the possibility that the inhibition of NO synthase could be used as a strategy to enhance the clinical efficacy of serotonergic antidepressants (Harkin et al., 2004). The present study attempts to investigate the participation of L-arginine-NO-cGMP pathway in the antidepressant activity of venlafaxine in FST in mice.

3.1.2. MATERIALS AND METHODS

3.1.2.1. Animals: Refer to Chapter 1 (1.2.1.)

3.1.2.2. Forced Swim Test (FST): Refer to Chapter 1 (1.2.2.1.)

3.1.2.3. Activity monitoring in animals: Refer to Chapter 2 (2.1.2.6.)

3.1.2.4. Drugs and treatment

The following drugs were used: venlafaxine (Panacea Biotec Ltd., Lalru, India), L-arginine (Loba-Chemie, Mumbai, India), methylene blue (S.D.-Fine Chem Ltd. Gujarat, India), 7-nitroindazole (7-NI) (Tocris Bioscience, Missouri, USA), sildenafil (Panacea Biotec, Lalru, India). All the drugs were dissolved in distilled water except 7-nitroindazole which was dissolved in few drops of Tween 80 and volume was made with distilled water. The doses of the drugs used were selected according to the previous studies conducted in our laboratory (Patil et al., 2005) and as reported in the literature (Harkin et al., 2004; Almeida et al., 2006). Different doses were administered intraperitoneally in a fixed volume of 1 ml/100g body weight 30 minutes before the animals were subjected to test. The possible participation of the L-arginine-NO-cGMP pathway in the antidepressant effect of venlafaxine was investigated. Mice were pretreated with L-arginine, a precursor of NO (750 mg/kg, i.p., a dose that produces no effect in the FST), or vehicle. Thirty minutes after L-arginine, venlafaxine (8 mg/kg, i.p., a dose active in the FST and having no effect
on the locomotor activity) or vehicle was injected and 30 minutes later animals were subjected to FST. In another set of experiments, we investigated the synergistic effect of venlafaxine (2 mg/kg., i.p, a subeffective dose) with a subeffective dose of 7-nitroindazole (25 mg/kg., i.p., a specific neuronal nitric oxide synthase inhibitor) or methylene blue (10 mg/kg, i.p., an inhibitor of NOS synthase and an inhibitor of sGC). These modulators were administered 30 minutes before venlafaxine or vehicle and 30 minutes later challenged with FST. To observe the role of cGMP in the antidepressant action of venlafaxine, mice received an injection of sildenafil (5 mg/kg., i.p, phosphodiesterase 5 inhibitor) or vehicle 30 min before venlafaxine (8 mg/kg., i.p.). Thirty minutes of venlafaxine administration the animals were subjected to FST. The same drug treatment schedule was followed while measuring the locomotor activity in mice. Different group of animals were taken for both tests.

3.1.2.5. Statistical analysis
Results expressed as mean (sec.) ± S.E.M and the data were analyzed using One-Way or Two-Way Analysis of Variance (ANOVA) wherever applicable. If any statistically significant change was found, post-hoc comparisons were performed using a Dunnett’s test. P<0.05 was considered statistically significant.

3.1.3. RESULTS
Venlafaxine, in a dose range of 4, 8 and 16 mg/kg produced a decrease in immobility period (in seconds) with respect to vehicle control group (Fig. 3.1.1.). Venlafaxine at the lower dose (2 mg/kg., i.p.) was ineffective in decreasing the immobility time in mice (Fig. 3.1.1.). Venlafaxine (2-8 mg/kg., i.p.) did not alter the locomotor activity in mice (Table 3.1.1.). However, venlafaxine at 16 mg/kg., i.p. dose increased the locomotor activity as evident from an increase in ambulatory movements (Table 3.1.1.). A dose of 8 mg/kg., i.p. (showing active in FST and not affecting
locomotor activity) or 2 mg/kg, i.p. (dose ineffective in FST) was chosen for carrying out drug interaction studies.

Fig. 3.1.1. Effect of different doses of venlafaxine (2, 4, 8 and 16 mg/kg) on the mean immobility period in mouse FST. The values are expressed as mean ± S.E.M. (n = 6-8). Data were analysed by One Way Analysis of Variance (ANOVA) followed by Dunnett’s test. *p < 0.001 compared with the vehicle-treated control. *p < 0.01 compared with the venlafaxine (2 mg/kg., i.p.) group. *p < 0.01 compared with the venlafaxine (4 mg/kg., i.p.) group. *p < 0.01 compared with the venlafaxine (8 mg/kg., i.p.) group.

Pretreatment with a subeffective dose of L-arginine (750 mg/kg., i.p., NO precursor) reversed the antidepressant action of venlafaxine (8 mg/kg., i.p.) as shown by an increase in immobility period compared to venlafaxine (8 mg/kg., i.p.) per se group [F=21.165, p<0.001] (Fig. 3.1.2). The combination did not affect the locomotor activity as compared to vehicle treated group (Table 3.1.1.).

Similarly, 7-nitroindazole (25 mg/kg., i.p., a specific nNOS inhibitor) enhanced the antidepressant effect of subeffective dose of venlafaxine (2 mg/kg., i.p.) [F=3.073, p<0.05] (Fig. 3.1.3.).
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Methylene blue (10 mg/kg., i.p.) did not affect the immobility period per se. However, methylene blue significantly enhanced the antidepressant effect of subeffective dose of venlafaxine (2 mg/kg., i.p.) \[F=10.906, p<0.001\] (Fig. 3.1.4.).

![Fig. 3.1.2. Effect of venlafaxine (8 mg/kg., i.p.) and its modification by L-arginine (750 mg/kg., i.p.) on the mean immobility period in mouse FST. L-arginine was administered 30 minutes before the treatment with venlafaxine and after further 30 minutes, animals were challenged with FST. The values are expressed as mean ± S.E.M. (n = 6–8). Data were analyzed by Two Way Analysis of Variance (ANOVA) followed by Dunnett’s test. *p < 0.001 compared with the vehicle-treated control group; **p < 0.001 compared with the same group pretreated with vehicle.

Fig. 3.1.5. shows the effect of pretreatment with sildenafil (5 mg/kg, i.p., a PDE5 inhibitor). Sildenafil did not affect immobility per se, but pretreatment with sildenafil reversed the antidepressant effect of venlafaxine \[F=28.078, p<0.001\].
Combination of all the NO modulators with venlafaxine did not affect the locomotor activity of mice (Table 3.1.1.).

Fig. 3.1.3. Effect of subeffective dose of venlafaxine (2 mg/kg, i.p.) and its modification by 7-nitroindazole (7-NI, 25 mg/kg, i.p.) on the mean immobility period in mouse FST. 7-NI was administered 30 minutes before the treatment with venlafaxine and after further 30 minutes, animals were challenged with FST. The values are expressed as mean ± S.E.M. (n = 6–8). Data were analyzed by Two Way Analysis of Variance (ANOVA) followed by Dunnett’s test. *p < 0.05 compared to 7-NI per se group.

3.1.4. DISCUSSION

L-arginine-NO-cGMP an important signaling pathway has been recently implicated in depression (Mantovani et al., 2003). In the present study, the antidepressant action of venlafaxine was attenuated by pretreatment with L-arginine and the action of subeffective dose of venlafaxine was potentiated by subeffective doses of 7-nitroindazole (neuronal nitric oxide synthase inhibitor) or methylene blue (NOS inhibitor and soluble guanylate cyclase inhibitor). Another important observation of the study was the
reversal of antidepressant action of venlafaxine by sildenafil (phosphodiesterase 5 inhibitor).

![Graph showing the effect of subeffective dose of venlafaxine and its modification by methylene blue on the mean immobility period in mouse FST.](image)

**Fig. 3.1.4.** Effect of subeffective dose of venlafaxine (2 mg/kg., i.p.) and its modification by methylene blue (10 mg/kg., i.p.) on the mean immobility period in mouse FST. Methylene blue was administered 30 minutes before the treatment with venlafaxine and after further 30 minutes, animals were challenged with FST. The values are expressed as mean ± S.E.M. (n = 6–8). Data were analyzed by Two Way Analysis of Variance (ANOVA) followed by Dunnett’s test. *p < 0.001 compared to methylene blue per se group.

The present study for the first time demonstrated the antidepressant-like effect of an acute administration of venlafaxine in the forced swim test (FST) is linked to modulating the L-arginine-NO-cGMP pathway.

The forced swimming-induced state of immobility in animals claimed to represent a condition similar to human depression (Renard et al., 2003) and amenable to reversal by antidepressant drugs (Kulkarni and Mehta, 1985). This model is widely accepted to screen antidepressant
drugs belonging to all major classes of antidepressants including tricyclics, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and atypical antidepressants (Porsolt et al., 1978a; 1978b).

**Fig. 3.1.5.** Effect of venlafaxine (8 mg/kg., i.p.) and its modification by sildenafil (5 mg/kg., i.p.) on the mean immobility period in mouse FST. Sildenafil was administered 30 minutes before the treatment with venlafaxine and after further 30 minutes, animals were challenged with FST. The values are expressed as mean ± S.E.M. (n = 6–8). Data were analyzed by Two Way Analysis of Variance (ANOVA) followed by Dunnett's test. *p < 0.001 compared to vehicle treated control group. **p < 0.001 compared with the same group pretreated with vehicle.

Nitric oxide (NO) plays a significant neuromodulatory role in the CNS. Any pharmacological manipulation of NO pathway may be considered as a novel therapeutic approach for the management of CNS disorders, more so for mental depression (Heiberg et al., 2002). Several in vivo studies have shown a modulatory role of NO in the extracellular levels of serotonin (5-HT) reuptake mechanism in the CNS (Harkin et al., 2003).
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Table 3.1.1. Effect of different dose of venlafaxine (2-16 mg/kg., i.p.) per se and its combination with L-arginine, 7-nitroindazole (7-NI), methylene blue, sildenafil on the locomotor activity measured for total of 5 minute session.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Dose (mg/kg., i.p.)</th>
<th>Mean ambulatory movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control</td>
<td>200 ± 8.72</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Venlafaxine</td>
<td>2</td>
<td>196 ± 6.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>212 ± 6.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>227 ± 6.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>284 ± 3.25*</td>
</tr>
<tr>
<td>3</td>
<td>L-arginine</td>
<td>750</td>
<td>187 ± 9.65</td>
</tr>
<tr>
<td>4</td>
<td>L-arginine + venlafaxine</td>
<td>750 + 8</td>
<td>216 ± 5.02</td>
</tr>
<tr>
<td>5</td>
<td>7-nitroindazole</td>
<td>25</td>
<td>215 ± 6.58</td>
</tr>
<tr>
<td>6</td>
<td>7-nitroindazole (7-NI) + venlafaxine</td>
<td>25 + 2</td>
<td>207 ± 6.36</td>
</tr>
<tr>
<td>7</td>
<td>Methylene blue</td>
<td>10</td>
<td>198 ± 3.65</td>
</tr>
<tr>
<td>8</td>
<td>Methylene blue + venlafaxine</td>
<td>10 + 2</td>
<td>190 ± 4.27</td>
</tr>
<tr>
<td>9</td>
<td>Sildenafil</td>
<td>5</td>
<td>186 ± 9.65</td>
</tr>
<tr>
<td>10</td>
<td>7-NI + sildenafil</td>
<td>5 + 8</td>
<td>185 ± 8.65</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± S.E.M. (n = 6-8). *p < 0.01 compared to vehicle treated control group.

The NO synthase inhibitors have been reported to possess antidepressant-like behavioral properties at doses that are without any effect on locomotor activity (Wegener et al., 2003). These effects are dose-dependent and stereoselective and can be reversed by co-treatment with the NO precursor, L-arginine. L-arginine is reported to exert a U-shape effect in the forced swim test, doses ranging from 30, 100 to 1000., mg/kg with lower dose causing no alteration, middle dose causing statistically significant reduction, and higher doses causing no alteration in the immobility period, respectively (Ergun and Ergun, 2007). We have chosen the dose of L-arginine (750 mg/kg., i.p.) that did not affect the immobility time and the locomotor activity per se. In our study, pretreatment of subeffective dose of L-arginine (750 mg/kg., i.p., NO precursor) resulted in the reversal of the antidepressant action of venlafaxine (8 mg/kg., i.p.) as shown by increased in immobility period as
compared to the action of venlafaxine (8 mg/kg., i.p.) per se group. Similarly, the antidepressant effects of imipramine were also blocked by pre-treatment with L-arginine and contrary to this, NO synthase inhibitor, N^G^-nitro-L-arginine (L-NA) augmented the behavioral effect of imipramine or fluoxetine in the forced swim test (Harkin et al., 2004). The studies carried out in our laboratory have demonstrated that fluoxetine suppressed the dependence and development of tolerance to the antinociceptive effect of morphine (Singh et al., 2003). Fluoxetine-induced suppression was potentiated by L-NAME and accentuated by L-arginine, thus demonstrating NO modulation of drug effects (Singh et al., 2003). These studies argue for the possibility of inhibition of NO synthase could be a strategy to enhance the clinical efficacy of serotonergic antidepressants.

Monoaminergic concept of antidepressant therapy has received support from the effective use of many drugs including venlafaxine which has dual reuptake inhibiting properties (Gould et al., 2006). In the present study, it was observed that venlafaxine produced dose-dependent reduction in immobility period in FST in mice. This effect was independent of any alterations in the locomotor activity. As expected, when the lowest dose of venlafaxine (2 mg/kg., i.p.) was combined with 7-nitroindazole or methylene blue it exhibited a synergistic effect. On the contrary, an inhibitory effect was observed when it (8 mg/kg., i.p.) was combined with L-arginine or sildenafil.

Previous reports have suggested that 7-nitroindazole (50 mg/kg), a specific neuronal NOS inhibitor and methylene blue (15 and 30 mg/kg), a direct inhibitor of both NOS and soluble guanylate cyclase (sGC) (Patil et al., 2005), inhibits the immobility time in FST. The effect is comparable to standard antidepressant drugs like imipramine (Eroglu and Caglayan, 1997; Volke et al., 2003). Similar studies have shown the anti-immobility effect of a specific inhibitor of sGC i.e. ODQ [1H-[1, 2, 4] Oxadiazole [4, 3-a] quinoxalin-1-one] (Heiberg et al., 2002; Ergun and Ergun, 2007) in FST and its immobility time can be reversed by pretreatment with L-arginine,
suggesting that NO-sGC pathway may play an important role in the mediation of its behavioral effect in the FST (Heiberg et al., 2002).

Thus, these results indicate that the inhibition of NO synthesis may underlie the reduction in the immobility time in the FST elicited by venlafaxine.

Another interesting observation of the present study was the reversal of the antidepressant-like effect of venlafaxine by sildenafil, a PDE5 inhibitor (Kulkarni and Patil, 2004). This indicates that venlafaxine exerts its effect in the FST probably by decreasing cGMP levels. The intracellular cGMP concentrations are regulated not only by sGC, but also by PDE5, which catalyses the hydrolysis of the second messengers cAMP and cGMP to yield AMP and GMP, respectively (Denninger and Marletta, 1999). The duration and magnitude of an NO-induced cGMP signal is determined by the activity of PDE5. PDE5 is expressed in several brain areas, particularly in the neurons of the Purkinje cell layer in the cerebellum and in the pyramidal neurons of the hippocampus (Bender and Beavo, 2004).

Taken together, the present study concluded the involvement of L-arginine-nitric oxide- cyclic GMP signaling pathway in the antidepressant effect of venlafaxine. Moreover, the present findings support the notion that the inhibition of NO production in the brain may be critical to the action of antidepressants.

Part-2: Involvement of sigma-1 receptor modulation in the antidepressant action of venlafaxine

3.2.1. INTRODUCTION

Various behavioral, biochemical and molecular studies are being carried out to elucidate the exact mechanism of antidepressant effect of venlafaxine. The molecule is known to exhibits six- to seven-fold selectivity for inhibition of serotonin reuptake as compared to
norepinephrine reuptake in synaptosome of rat brain and a 15- to 30-fold higher affinity for SERT binding sites as compared to those of NET (Gould et al., 2006). In a study mentioned above, we have demonstrated the involvement of L-arginine-nitric oxide-cyclic guanosine monophosphate signaling pathway in mediating the antidepressant activity of venlafaxine in mouse FST. It was suggested that nitric oxide synthase (NOS) inhibitors when administered prior to venlafaxine treatment enhances its antidepressant-like action in mouse forced swim test (FST). Also, some of the centrally-mediated benefits of venlafaxine in depression may be due to its intracellular properties especially on the neuro-glial circuitry and MAPK/p90Rsk-dependent pathways at an early stage (Khawaja et al., 2003).

Sigma receptors have recently been the target of drug development related to psychiatric disorders, including schizophrenia, depression and Alzheimer's (Ishihara and Sasa, 2002). Out of the established two isoforms of sigma receptors (subtypes sigma-1 and sigma-2), sigma-1 receptors are particularly involved in learning and memory, response to stress and depression, psychostimulant-induced sensitization, vulnerability to addiction and pain perception (Hashimoto and Ishiwata, 2006). Sigma-1 receptor agonists when administered in mice or rat modulate the release of serotonin and dopamine in the brain (Kobayashi et al., 1997; Bermack and Debonnel, 2005). Further, it was suggested that stimulation of sigma-1 receptors alleviate behavioral despair in both forced swim test (FST) (Skuza and Rogoz, 2006) and tail suspension test (TST) (Ukai et al., 1998). Sigma receptors may play, in some way, a role in the antidepressant actions of selective serotonin reuptake inhibitors such as fluoxetine, fluvoxamine or escitalopram (Narita et al., 1996). One of the earlier studies from our laboratory has indicated the involvement of sigma-1 receptors in modulating the antidepressant-like effect of neurosteroids in mouse FST (Reddy et al., 1998).
As sigma-1 receptors are known to affect the release of catecholamines, affects the outcome of antidepressants, it was hypothetized that sigma receptor modulation may be involved in the antidepressant outcome of venlafaxine. With this background, the present study was conducted to expound the possible involvement of central sigma-1 receptors in the antidepressant-like action of venlafaxine in mouse FST. This was examined using (+) - pentazocine (a sigma-1 receptor agonist), progesterone (a sigma-1 receptor antagonist neurosteroid), rimcazole (another sigma-1 receptor antagonist) or BD 1047 (a novel sigma-1 receptor antagonist), respectively.

3.2.2. MATERIALS AND METHODS

3.2.2.1. Animals: Refer to Chapter 1 (1.2.1.)

3.2.2.2. Forced Swim Test (FST): Refer to Chapter 1 (1.2.2.1.)

3.2.2.3. Measurement of locomotor activity: Refer to Chapter 2 (2.1.2.6.)

3.2.2.4. Drugs and treatment

The following drugs were used: venlafaxine hydrochloride (Panacea Biotec, Lalru, India), (+) pentazocine (Ranbaxy Co., Gurgaon, India), progesterone (Sigma Aldrich Co., MO, USA), BD 1047 (Tocris Co., Missouri, USA), rimcazole (Sigma Aldrich Co., MO, USA). All the drugs except progesterone were dissolved in distilled water and different doses were administered intraperitoneally in a fixed volume of 0.1ml per 10g of body weight. Progesterone was made in vegetable oil and administered subcutaneously. Venlafaxine was administered 30 minutes before challenging the animals to FST. In case of interaction studies, various agonists or antagonists were given 15 minutes before administering venlafaxine. The experimental protocol comprised of various groups, each consisting of six to eight animals. Different set of animals were used for
measuring the locomotor activity. All the doses were chosen according to the literature available (Reddy et al., 1998; Skuza and Rogoz, 2003).

3.2.2.5. Statistical analysis

Results expressed as mean (s) ± S.E.M and the significance of the difference in the responses of treatment groups in comparison to the control was determined by One Way Analysis of Variance (ANOVA) followed by Tukey’s test. P<0.05 was considered statistically significant.

3.2.3. RESULTS

3.2.3.1. Effect of pretreatment of venlafaxine on forced swim test

Venlafaxine at different doses (4, 8 and 16 mg/kg., i.p.) produced dose dependent decrease in the immobility period (in seconds) with respect to the vehicle control group in forced swim test (Fig. 3.2.1A.). Venlafaxine (2 mg/kg., i.p.) was ineffective in affecting immobility period (Fig. 3.2.1A).

Venlafaxine (2-8 mg/kg., i.p.) did not alter the locomotor activity in mice. However, venlafaxine at 16 mg/kg., i.p. dose increased the locomotor activity as evident from an increase in ambulatory movements. [Ambulatory movements - Control : 200 ± 8.72, Venlafaxine (2 mg/kg) 196 ± 6.24, Venlafaxine (4 mg/kg) 212 ± 6.95, Venlafaxine (8 mg/kg) 227 ± 6.21, Venlafaxine (16 mg/kg) : 284 ± 3.25] (One Way Analysis of Variance followed by Tukey’s test). A dose of 8 mg/kg., i.p. (showing active in FST and not affecting locomotor activity) or 2 mg/kg., i.p. (dose ineffective in FST and not affecting locomotor activity) was chosen for carrying out drug interaction studies.

3.2.3.2. Effect of various sigma receptor modulators on the action of venlafaxine in FST

To assess the sigma modulatory action of venlafaxine, it was combined with (+) pentazocine, a sigma-1 receptor agonist. The subeffective dose of (+) pentazocine (2.5 mg/kg., i.p.) enhanced the antidepressant effect of subeffective dose of venlafaxine (2 mg/kg., i.p.) (Fig. 3.2.1B.).
Pretreatment with a subeffective dose of progesterone (10 mg/kg., s.c., sigma-1 receptor antagonist neurosteroid), rimcazole (5 mg/kg., i.p., sigma-1 receptor antagonist) or BD 1047 (1 mg/kg., i.p., novel sigma-1 receptor antagonist), reversed the antidepressant action of venlafaxine (8 mg/kg., i.p.) as shown by an increase in immobility period compared to venlafaxine (8 mg/kg., i.p.) per se group (Fig. 3.2.2.).

*Fig. 3.2.1. (A) Effect of different doses of venlafaxine (2, 4, 8 and 16 mg/kg., i.p.) (B) and its modification by (B) (+) pentazocine (2.5 mg/kg., i.p.) on the mean immobility period (seconds) in mouse FST. Venlafaxine was administered 30 minutes before challenging the animals to FST. Pentazocine was administered 15 minutes before the treatment with venlafaxine and after further 30 minutes, animals were challenged with FST. The values are expressed as mean ± S.E.M. (n = 6–8). Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey’s test. *p < 0.001 compared with the vehicle-treated control. *p < 0.001 compared with the venlafaxine (2 mg/kg., i.p.) group; #p < 0.001 compared with the pentazocine + venlafaxine (2 mg/kg., i.p.) group; $p < 0.01 compared with the venlafaxine (4 mg/kg., i.p.) group; §§p < 0.01 compared with the venlafaxine (8 mg/kg, i.p.) group, V: Vehicle.
Fig. 3.2.2. Effect of venlafaxine (8 mg/kg, i.p.) and its modulation by progesterone (10 mg/kg, s.c.), rimcazole (5 mg/kg, i.p.) or BD 1047 (1 mg/kg, i.p.) on the immobility period (seconds) induced by FST. Various antagonists were administered 15 minutes before venlafaxine (8 mg/kg, i.p.) and after 30 minutes of venlafaxine injection, mice were challenged to FST. Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey's test. *p<0.05 as compared to vehicle treated group. **p<0.05 as compared to venlafaxine (8 mg/kg, i.p.) treated group. V: Vehicle.
3.2.4. DISCUSSION

In the present experiment, venlafaxine at different doses (4-16 mg/kg., i.p.) decreased the immobility period in mouse forced swim test. The anti-immobility action of venlafaxine was not associated with the change in the locomotor activity. Sigma receptor agonist potentiated the outcome of subeffective dose of venlafaxine. Various sigma receptor antagonists particularly the sigma-1 subtype reversed the antidepressant action of venlafaxine. This is the first study which demonstrated the involvement of sigma-1 receptor modulation in mediating the anti-immobility effect of venlafaxine in mouse forced swim test.

In the present study, venlafaxine reduced the immobility time in mouse FST without any effect on the locomotor activity.

Sigma receptors are non-opioid, non-phencyclidine receptors that contain two subtypes: sigma-1 and sigma-2 receptors. Sigma-1 receptors are expressed in specific regions of the brain such as layers of the cortex, hippocampus, hypothalamic nuclei, substantia nigra and purkinje cells in the cerebellum (Heroux et al., 1992; Kitaichi et al., 2000) and have recently been the target of drug development related to psychiatric disorders including schizophrenia and depression (Ishihara and Sasa, 2002). Many pharmacological and physiological actions have been attributed to sigma-1 receptors. Sigma-1 receptors modulate the release of various neurotransmitters (Su and Hayashi, 2003) like serotonin (Bermack and Debonnel, 2005), dopamine (Kobayashi et al., 1997) or glutamate (Yagasaki et al., 2006) and known to alleviate the symptoms of depression. Sigma-1 agonists have been then tested in various behavioral tests classically used to predict an antidepressant activity. SA 4503, (+)-pentazocine, DTG, JO-1784, and SKF-10,047 (all sigma-1 receptor agonist) dose dependently decrease immobility in the FST (Matsuno et al., 1996a; Kinsora et al., 1998; Skuza and Rogoz, 2003). These effects were blocked by the sigma-1 receptor antagonists, NE-100 or BD1047. Further, SA 4503 and (+)-pentazocine (both sigma-1 receptor agonists) also decreased immobility period in the tail-suspension test, an effect also antagonized by NE-100 (Ukai et al., 1998).
Many antidepressant drugs interact with sigma receptors and accumulating evidence suggests that these proteins mediate antidepressant-like effects in animals and humans (Wang et al., 2007). Various antidepressants such as fluvoxamine, fluoxetine, citalopram, sertraline, clorgyline, and imipramine all possess moderate to high affinity for sigma-1 sites (Bermack and Debonnel, 2005) and it has been proposed that certain differences in the clinical effects of various antidepressants may, in part, be explained by their distinct influence on cerebral sigma receptors. Selective serotonin reuptake inhibitors (SSRIs) are known to have high affinities for sigma-1 receptors, while tricyclic antidepressants (TCAs) have less (Narita et al., 1996; Takebayashi et al., 2004). Fluvoxamine shows the utmost potent effectiveness in the treatment of psychotic depression and has the highest affinity for sigma-1 receptors among SSRIs (Narita et al., 1996; Stahl, 2005). In one of the studies, progesterone and BD 1047 (a sigma-1 receptor antagonist) counteracted the antidepressant-like effect induced by co-administration of pramipexole and sertraline (Rogoz and Skuza, 2006). Sigma-1 receptors are also known to up-regulate the release of BDNF (brain-derived neurotrophic factor) which in turn has been suggested to contribute to the action of antidepressants (Yagasaki et al., 2006). The sigma ligands are hypothesized to increase the firing activity of Dorsal Raphe Nucleus (DRN). Treatment with sigma ligands may rapidly modulate N-methyl-D-aspartate (NMDA) receptor-mediated transmission in the hippocampus, and potentially other forebrain regions, which in turn would lead to a modulation of 5-HT neurotransmission in the DRN via feedback loops (Bermack and Debonnel, 2005). Sertraline (selective serotonin reuptake inhibitor) or clorgyline (monoamine oxidase inhibitor) potentiate the NMDA response on pyramidal neurons in the CA3 region of the rat dorsal hippocampus with a bell-shaped dose response curve, which is reversed by haloperidol (a sigma-1 receptor antagonist) (Bergeron et al., 1993). Further, combined treatment with sigma ligands and amantadine or memantine (NMDA receptor antagonist) may be an alternative to the
treatment of antidepressant-resistant depressive patients in the future (Skuza and Rogoz, 2006). Although, various studies have reported the involvement of sigma receptors in the action of various antidepressants, none of them have explored the involvement of this receptor system in the antidepressant outcome of venlafaxine.

Venlafaxine, the first dual reuptake inhibitor of serotonin and norepinephrine, is indicated in major depressive disorder, generalized anxiety disorder, social anxiety disorder, and panic disorder (Lee and Keltner, 2006). It is also known to hinder most behavioral effects of sleep deprivation and is associated with interference of catecholamine release in the striatum (De Oliveira et al., 2004). Also, sustained treatment with venlafaxine increases activity-related cytoskeletal protein (Arc) expression in corticolimbic regions (Pei et al., 2004). Up-regulation of Arc expression may be part of the process by which antidepressant drugs achieve long-term changes in synaptic function in the brain (Pei et al., 2003).

To elucidate the interaction of venlafaxine with sigma receptors, (+) pentazocine, a selective sigma-1 receptor agonist was used and it was found that pretreatment with subeffective dose (the dose which did not affect the immobility period in FST) potentiated the antidepressant activity of subeffective dose of venlafaxine. Further, the antidepressant effect of effective dose of venlafaxine (a dose devoid of any effect on locomotor activity) is antagonized by progesterone, a sigma-1 receptor antagonist neurosteroid, rimcazole, a sigma-1 receptor antagonist antipsychotic agent, or BD 1047, a novel selective sigma-1 receptor antagonist.

There may be possibility of some pharmacokinetic interaction between the venlafaxine and sigma receptor modulators. However, the protein-binding ability of venlafaxine is very low, being less than 30%, the chances of pharmacokinetic interaction is less (Wiklander et al., 1995).

In conclusion, these findings suggest the participation of sigma receptors (sigma-1 site) in the anti-immobility effect of venlafaxine in mouse forced swim test.
Part-3: Positive modulation by yohimbine ($\alpha_2$ adrenoceptor antagonist) of the antidepressant activity of fluoxetine or venlafaxine

3.3.1. INTRODUCTION
Recent clinical trials have shown the usefulness of yohimbine, a $\alpha_2$ receptor antagonist in patients suffering from panic disorder and agoraphobia. The plasma levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), a principal metabolite of brain norepinephrine (NE), in these patients were significantly increased following the treatment with yohimbine, thus confirming the noradrenergic modulation (Charney et al., 1984). The alpha (2A)-adrenoceptors knock-out mouse was found to be less active in a Porsolt's forced swim test and reported to be insensitive to the effects of the tricyclic antidepressant imipramine (Schramm et al., 2001). More evidences have suggested that yohimbine also modulates the serotonergic system in the brain and the addition of yohimbine was found to have a beneficial interaction with fluoxetine (a selective serotonin reuptake inhibitor, SSRI) (Sanacora et al., 2004). The outcome of the clinical study was supported by the various microdialysis studies, suggesting that $\alpha_2$-adrenoceptors profoundly influence the serotonergic firing rates also (Sanacora et al., 2004). In another study, the administration of yohimbine (5 mg/kg, i.p.) increased the extracellular levels of serotonin (5-HT) in the rat frontal cortex by approximately 230% of the basal levels (Cheng et al., 1993). Yohimbine also augmented the effect of fluvoxamine in refractory depressed patients (Cappiello et al., 1995). The earlier studies carried out in our laboratory have shown yohimbine to reverse clonidine-induced behavioral despair in mice. Systemically administered clonidine produced depression either by inhibiting the firing of brain noradrenaline neurons by acting directly upon adrenergic receptors located on or near the soma of these neurons or through concomitant inhibition of 5-HT neurons in an indirect manner.
(possibly secondary to an impairment in noradrenergic transmission)
(Svensson et al., 1975; Parale and Kulkarni, 1986).

Yohimbine has also been found to be potential agent for the
treatment of fluoxetine-induced sexual dysfunction (Jacobsen, 1992;
Balon, 1993). Considering the inhibitory effects of the $\alpha_2$-adrenergic
receptor on serotonergic function, it was hypothesized that the addition of
a $\alpha_2$-antagonist to SSRI or SNRI treatment regimen may result in
enhanced postsynaptic serotonergic/adrenergic transmission. With this
background, the present study was conducted to examine the combined
effect of yohimbine with two of the widely used antidepressants namely
fluoxetine (selective serotonin reuptake inhibitor, SSRI) or venlafaxine
(dual reuptake inhibitors of both serotonin and norepinephrine, SNRI) in an
animal model of depression.

3.3.2. MATERIALS AND METHODS

3.3.2.1. Animals: Refer to Chapter 1 (1.2.1.)

3.3.2.2. Forced Swim Test (FST): Refer to Chapter 1 (1.2.2.1)

3.3.2.3. Measurement of locomotor activity: Refer to Chapter 2
(2.1.2.6.)

3.3.2.4. Drugs: The drugs used in the present study were obtained from
following drug houses: fluoxetine (Eli Lilly & Co. Indiana, USA),
venlafaxine (Panacea Biotec Ltd. Lalru, India), yohimbine (Sigma Aldrich
Co., MO, USA). All the drugs were dissolved in distilled water and different
doses were administered intraperitoneally in a fixed volume of 1 ml/100g
body weight. Fluoxetine or venlafaxine at different doses were
administered 30 minutes before the animals were subjected to forced
swim test. In combination study, yohimbine was administered 15 minutes
before fluoxetine or venlafaxine and after 30 minutes of fluoxetine or
venlafaxine treatment, animals were challenged with forced swim test.
3.3.2.5. Statistical analysis

Results expressed as mean (sec.) ± S.E.M and the significance of the difference in the responses of treatment groups in comparison to the control was determined by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. P<0.05 was considered statistically significant.

3.3.3. RESULTS

Fluoxetine, when used in a dose range of 5, 10, 20 and 40 mg/kg resulted in the decrease of immobility period (in seconds) with respect to vehicle control group (control: 211.0 ± 3.27, 5 mg/kg: 206.0 ± 7.55, 10 mg/kg: 101.0 ± 26.35: 20 mg/kg: 56.50 ± 14.01, 40 mg/kg: 26.0 ± 17.78) [F=28.352] (Fig. 3.3.1.). Also, fluoxetine (5-40 mg/kg., i.p.) did not alter the locomotor activity in mice at all the doses tested. Yohimbine (2 mg/kg., i.p.) was ineffective in reducing the immobility period in forced swim test. However, pretreatment with yohimbine enhanced the antidepressant action of fluoxetine at 5, 10 and 20 mg/kg dose (Fig. 3.3.1.). At higher dose of fluoxetine (40 mg/kg., i.p.), administration of yohimbine (2 mg/kg., i.p.) did not produce synergistic effect (Fig. 3.3.1.). However, yohimbine enhanced the anti-immobility action of subeffective dose of fluoxetine (5 mg/kg., i.p.) (Fig.3.3.1). The combination of yohimbine with different doses of fluoxetine did not affect the locomotor activity in mice as compared to fluoxetine per se effect (Table 3.3.1.).

Similarly, venlafaxine, when used in a dose range of 2, 4, 8 and 16 mg/kg resulted in a decrease in immobility period (in seconds) with respect to vehicle control group (Control: 157.75 ± 8.34, 2 mg/kg: 147.50 ± 12.51, 4 mg/kg: 85.33 ± 15.10: 8 mg/kg: 84.17 ± 14.89, 16 mg/kg: 33.50 ± 7.58) [F=17.842] (Fig. 3.3.2.). Venlafaxine (2-16 mg/kg., i.p.) did not alter the locomotor activity in mice at all the doses tested. Pretreatment with yohimbine enhanced the antidepressant action of venlafaxine at 2, 4 and 8 mg/kg dose, respectively. At higher dose of venlafaxine (16 mg/kg., i.p.), addition of yohimbine (2 mg/kg., i.p.) did not produce synergistic effect (Fig. 3.3.2.). However, yohimbine enhanced the anti-immobility action of
subeffective dose of venlafaxine (2 mg/kg., i.p.) (Fig.3.3.2.). The combination of yohimbine with different doses of venlafaxine did not affect the locomotor activity in mice as compared to venlafaxine per se (Table 3.3.1.).

**Fig. 3.3.1.** Effects of fluoxetine and its modulation by yohimbine on the immobility period during a 6 minute forced swim test in mice. Fluoxetine (5-40 mg/kg., i.p.) was administered 30 minutes before the swim test. In combination study, yohimbine was administered 15 minutes before fluoxetine and after 30 minutes of fluoxetine treatment, animals were challenged with forced swim test. Values are expressed as mean ± SEM (n=6-8 animals per group). Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. #p<0.05 as compared to vehicle treated group. *p<0.05 as compared to respective fluoxetine treated group.

### 3.3.4. DISCUSSION

The main findings of this study are consistent with the hypothesis postulating that co-administration of $\alpha_2$-agonist yohimbine can hasten the antidepressant effects of SSRI and SNRI medications. In the present study, we had chosen fluoxetine, a selective serotonin reuptake inhibitor (SSRI) and venlafaxine, dual reuptake inhibitor of serotonin and
norepinephrine (SNRI) for interaction studies. Preclinical studies of venlafaxine have suggested that at lower plasma concentration venlafaxine have higher affinity for the serotonin transport site which results in an action similar to fluoxetine. At higher concentration this molecule has also some affinity for the norepinephrine transport site (Redrobe et al., 1998). The doses used in the study were chosen based on our earlier studies and as reported in literature (Kulkarni and Mehta, 1985; Redrobe et al., 1998; Dhingra and Sharma, 2006).

**Fig. 3.3.2.** Effects of venlafaxine and its modulation by yohimbine on the immobility period during the 6 minute forced swim test in mice. Venlafaxine (2-16 mg/kg i.p.) was administered 30 minutes before the swimming test. In combination study, yohimbine was administered 15 minutes before venlafaxine and after 30 minutes of venlafaxine treatment, animals were challenged with forced swim test. Values are expressed as mean ± SEM (n=6-8 animals per group). Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett’s test. *p<0.05 as compared to vehicle treated group. **p<0.05 as compared to respective venlafaxine treated group.
The $\alpha_2$-adrenoceptors are present both pre- and postsynaptically in the central and peripheral nervous system and they are inhibitory in nature. Presynaptic $\alpha_2$ receptors participate in the negative feedback control of norepinephrine release. Postsynaptic $\alpha_2$ receptors are located mainly on liver cells, platelets, and the smooth muscle of blood vessels (Brannan et al., 1991). Yohimbine, essentially an $\alpha_2$ adrenoceptor antagonist could modulate the serotonergic system as well. It was observed that following the administration of yohimbine the levels of norepinephrine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5HIAA) were increased significantly in the lateral ventricular fluid of rats. These increases were abolished when animals were pretreated with alpha-methyl-para-tyrosine or reserpine. These findings indicate that yohimbine promotes NE and 5-

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Dose (mg/kg., i.p.)</th>
<th>Mean count (Ambulations + rearing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>-</td>
<td>200 ± 8.72</td>
</tr>
<tr>
<td>2</td>
<td>Fluoxetine</td>
<td>5</td>
<td>206 ± 4.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>204 ± 3.25</td>
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<tr>
<td></td>
<td></td>
<td>20</td>
<td>221 ± 3.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>226 ± 7.24</td>
</tr>
<tr>
<td>3</td>
<td>Fluoxetine + Yohimbine</td>
<td>5 + 2</td>
<td>201 ± 5.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 + 2</td>
<td>213 ± 10.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 + 2</td>
<td>209 ± 12.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 + 2</td>
<td>214 ± 6.17</td>
</tr>
<tr>
<td>4</td>
<td>Venlafaxine</td>
<td>2</td>
<td>196 ± 6.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>212 ± 6.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>227 ± 6.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>284 ± 3.25*</td>
</tr>
<tr>
<td>5</td>
<td>Venlafaxine + Yohimbine</td>
<td>2 + 2</td>
<td>192 ± 6.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 + 2</td>
<td>208 ± 11.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 + 2</td>
<td>215 ± 12.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 + 2</td>
<td>247 ± 6.67*</td>
</tr>
</tbody>
</table>

*p<0.05 as compared to vehicle treated group.
HT release in the brain (Brannan et al., 1991). Previous pharmacological studies have suggested that the firing activity of serotonin (5-HT) cells of the dorsal raphe nucleus is dependent on a tonically active, central adrenergic system. The $\alpha_2$ heteroreceptors located on presynaptic serotonergic terminals are tonically activated by endogenous noradrenaline (Mongeau et al., 1993; Feuerstein et al., 1993). Activation of $\alpha_2$-adrenergic auto- and heteroreceptors are known to decrease 5-HT transmission. In addition, inhibitory $\alpha_2$-autoreceptors found on norepinephrine neurons in the locus ceruleus decrease firing of these neurons and thus decrease serotonergic transmission by reducing activation of stimulatory $\alpha_1$-receptors located on the 5-HT cell bodies in the raphe nucleus (Svensson et al., 1975; Baraban et al., 1980). Systemic administration of $\alpha_2$-antagonists has been shown to enhance serotonergic neurotransmission via direct inhibition of the $\alpha_2$-heteroreceptors located on the serotonergic nerve terminals and indirect stimulation of $\alpha_1$-receptors via inhibition of $\alpha_2$-autoreceptors (Freedman and Aghajanian, 1984; Haddjeri et al., 1996; Hopwood and Stamford, 2001). In the present study, the addition of yohimbine at a dose which is subeffective in reducing the immobility period in mice when added to different doses of fluoxetine (SSRI) or venlafaxine (SNRI) resulted in enhanced activity as compared to their effect per se.

The results of the present study suggested that the antidepressant effect of fluoxetine or venlafaxine could be facilitated (potentiated) by concomitant use of yohimbine. However, the pharmacokinetic interactions between and yohimbine and fluoxetine or venlafaxine needs to be understood for explaining the interactions.