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Antidepressant like Effect of N(G)-Nitro-L-Arginine Methyl Ester

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Abstract: The aim of the present study was to evaluate the antidepressant action of Nitric Oxide (NO) synthase inhibitor L-NAME (N(G)-nitro-L-arginine methyl ester) as well as its interaction with the conventional antidepressant drugs and to delineate the possible mechanism of its antidepressant action using forced swimming model in mice. Also, synergistic action of L-NAME and the drugs which act on the receptors involved in depression was studied. The antidepressant activity was assessed in Forced Swim Test in S.Mice. Total immobility period was recorded during a 6 min time. The L-NAME (10 mg kg^{-1}, i.p.) produced a reduction in immobility period in force Swim Test. L-NAME (10 mg kg^{-1}, i.p.) reversed the clonidine (150 μg kg^{-1}, i.p.) induced depressions in mice were observed. Clonidine induced increased immobility time was significantly reverse by the treatment with L-LAME. Whereas reserpine induced depression is significantly reversed by L-NAME. Pretreatment of haloperidol (2 mg kg^{-1}, i.p.) shown high immobility time which partially reversed by treatment of L-NAME. Pindolol (10 mg kg^{-1}, i.p.) fully block the antidepressant activity of L-NAME. Antidepressant-like effect of L-NAME (5 mg kg^{-1}) prevented by pretreatment with L-arginine in mice Force Swimming Test (FST). In addition, treatment of mice with methylene blue (10 mg kg^{-1} i.p.) (direct inhibitor of both Nitric Oxide Synthase (NOS) and guanylate cyclase) potentiates the effect of L-NAME (2.5 mg kg^{-1}) in FST. From above results it is concluded that the L-NAME produced antidepressant-like activity through adrenergic system and L-arginine-NO-cGMP pathway.

Key words: Nitric oxide synthase, antidepressant, forced swimming test, N (G)-nitro-L-arginine methyl ester

INTRODUCTION

Depression is most common illness that affects a large number of individuals. Depression is emphasized by finding its place in global burden of diseases. Depression was the fourth largest cause of burden of diseases worldwide in 1990 and by 2020 it is expected to be the second largest cause of burden of diseases (Lopez and Murray, 1996). The range of lifetime risk for major depressive disorder in community samples is from 10 to 20% for women and 5 to 12% for men (Janicak, 2001).

Within the last 50 years, specific antidepressant drugs have replaced early treatment of depression, this can be considered major progress. The marketed antidepressant drugs have a characteristic lag of onset of their therapeutic effect, post treatment relapses are frequent and a significant proportion of patients are not helped by available antidepressants, with efficacy being a particular concern in children and adolescents (Harro, 2004). It was useful for finding of more novel antidepressant drugs. The NOS inhibitors emerged as a new target for antidepressant therapy.

Nitric Oxide (NO) plays a significant neuromodulatory role in the Central Nervous System (CNS). Any pharmacological manipulation of NO pathway may be considered as a novel therapeutic approach for the management of CNS disorders (Heiberg *et al*., 2002). Several *in vivo* studies have shown a modulatory role of NO in the extracellular levels of serotonin reuptake mechanism in the CNS (Harkin *et al*., 2003). The Nitric Oxide Synthase (NOS) inhibitors have been reported to possess antidepressant-like behavioral properties at doses that are without any effect on locomotor activity (Wegener *et al*., 2003). The NO, a messenger molecule in the brain, synthesized from L-arginine by 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 (Joca and Guimaraes, 2006). The NO 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*., 2005).

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The L-NA (N(G)-nitro-1-arginine) and 7-NI (7-nitroindazole), dose dependently reduced immobility in the rat Force Swim Test (FST) (Harkin *et al*., 2003). It means there was possibility of inhibition of NOS could be used as a strategy to enhance the clinical efficacy of antidepressants (Harkin *et al*., 2004). The 7-NA and 7-NI also used as strategy of serotonergic antidepressant (Yildiz *et al*., 2000). The N(G)-nitro-L-arginine (L-NNA) potentates the effect of a sub effective dose of memantine which produced an antidepressant-like effect in the FST that seems to be mediated through an interaction with the L-arginine-NO-cGMP pathway (Volke *et al*., 2003). The 1-(2-trifluoromethylphenyl)-imidazole (TRIM), a novel neuronal Nitric Oxide Synthase (nNOS) inhibitor augments the effects of antidepressants acting on serotonergic system in the FST (Ulak *et al*., 2008).

The N (G)-nitro-L-arginine methyl ester (L-NAME) is a non-specific NOS inhibitor (Coskun *et al*., 2005). Previous studies with L-NAME show that it has antidepressant like action. But very little information is available on it.

MATERIALS AND METHODS

Animals: The project was conducted in the Wockhardt Research Centre, Aurangabad, Maharashtra, India. The duration of the project was from June 2008 to Jan. 2009. Male Swiss albino mice weighing between 25-30 g bred in Animal House facility of the Wockhardt Research Centre, Aurangabad, India. The animals were housed under standard laboratory conditions and maintained on 12/12 h light and dark cycle (lights on at 08:00 h) and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 09:00 and 15:00 h. The experimental protocols were approved by the Wockhardt Animal Ethics Committee (WAEC). Each group consists of 6-8 male Swiss albino mice.

Drug and treatment: The following drugs were used: L-NAME, Pindolol, Haloperidol, Methylene blue, Clorgiline (All from Sigma Aldrich, St. Louis, MO, USA.), Reserpine (Lancaster Synthesis, England), Clonidine (UNICHEM Laboratories Ltd., India,) L-Arginine and Venlafaxine (Wokhardt Ltd., India), Imipramine (Nicholas Piramal India Ltd.), Fluoxetine (Sun Pharma Ltd., India) and Chlorpromazine (LA Pharmaceutical Ltd.).

The L-NAME, L- arginine, methylene blue, clorgiline and chlorpromazine were dissolved in 0.9% normal saline. Pindolol, imipramine, fluoxetine, venlafaxine and chlorpromazine were dissolved in few drops of tween 80 and volume was made with 0.9% normal saline, while other drugs like haloperidol, clonidine and reserpine were dissolved in 1% DMSO. Control animals received, respective vehicles.

Antidepressant like activity of L-NAME was evaluated in forced swim test in Swiss mice. The L-NAME was administered at dose of 2.5, 5, 10 and 20 mg kg^{-1} by intraperitonial route. Observation was made at 1 and 3 h after L-NAME administration. Imipramine (10 mg kg^{-1}) was used as reference positive control.

In separate series of experiment, we investigated the interaction of L-NAME with agonist and or antagonist of different receptors involved in the depression such as clonidine ($alpha_2$ adrenoceptor agonist), reserpine (vesicular re-uptake blocker), haloperidol ($dopamine_2$ receptor agonist), pindolol (5- $HT_{1A/1B}$ receptor/ beta adrenoceptor antagonist), L- arginine (precursor of nitric oxide) and methylene blue (direct inhibitor of both NOS and soluble Guanylate Cyclase (sGC)).

To this end, mice were pretreated with clonidine (150 μg kg^{-1}), haloperidol (2 mg kg^{-1}), pindolol (20 mg kg^{-1}), L-arginine (750 mg kg^{-1}), methylene blue (10 mg kg^{-1}) and after 15 min L-NAME (10 mg kg^{-1}) was administered, whereas reserpine (2 mg kg^{-1}) was administered 4 h before L-NAME (10 mg kg^{-1}) administration. The animals thereafter subjected to forced swim test at 1 and 3 h post L-NAME administration. The doses of the some drugs used were selected according to previous studies conducted in our laboratory and as reported in the literature (Dhir and Kulkarni, 2007).

In another set of experiment, we investigated interaction of L-NAME with standard antidepressant drugs such as imipramine (dual reuptake inhibitor), fluoxetine Selective Serotonin Reuptake Inhibitor (SSRI), venlafaxine (dual reuptake inhibitors of serotonin and nor epinephrine) and clorgyline (selective monoamine oxidase-A inhibitor). An attempt was made to observe synergistic effect if any, of L-NAME with standard antidepressant drugs. For this purpose L-NAME and all other antidepressants were administered simultaneously at their sub-effective doses. Doses studied were L-NAME (2.5 mg kg^{-1}), imipramine (2.5 mg kg^{-1}), fluoxetine (5 mg kg^{-1}), venlafaxine (2 mg kg^{-1}) and clorgyline (125 μg kg^{-1}). The doses of the drugs used were selected according to earlier studies conducted in our laboratory and as reported in the literature (Dhir and Kulkarni, 2007).

All the drugs used in this study were administered by intraperitonial route in a constant volume of 10 mL kg^{-1}.

Forced swimming test: The test was conducted using the method of (Porsolt *et al*., 1977; Kulkarni and Mehta,

1985; Parale and Kulkarni, 1986). Briefly, mice were individually forced to swim in a glass jar (height: 25 cm; diameter: 10 cm) containing 15 cm of water maintained at 25±1°C. After the initial 2-3 min of vigorous activity, the animal showed period of immobility by floating with minimum movements. An animal considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose above the water surface. The total duration of immobility was recorded with the help of stop watch during next 4 min of a total 6 min test.

Locomotors activity: The assessment of locomotor activity was carried out on mice. Briefly, the locomotor activity (ambulation) of the mice was measured by a computerized LE 8811 IR Motor Activity Monitor (Panlab s.l.). This system is used to measure motor activity in experimentation with animals and it is based on a grid of infrared cells, that make it possible to determine the magnitude of motor activity on the basis of analysis of the position and frequency with which the experimental animal break the infrared breams. Mice were placed in the IR frame of 45×45 cm containing total of 16×16 infrared beams at an interval of 2.5 cm, located on the sides. Mice were placed in IR frame contacting cage, 1 min prior to the evaluation for acclimatization and then locomotion counts were recorded for a period of 10 min. The L-NAME was administered by intraperitonial route at dose of 5, 10 and 20 mg kg^{-1}. Chlorpromazine (10 mg kg^{-1}) was used as a reference positive control. All the drugs used in this study were administered by intraperitonial route in a constant volume of 10 mL kg^{-1}.

Statistical analysis: Results expressed as Mean±SEM and the data were analyzed by one-way or two-way analysis of variance (ANOVA) followed by post-hoc Bonferroni test wherever appropriate. Differences with $p<0.05$ was considered statistically significant. The ED_{50} were analyzed by Finneys Probit Analysis Method. (Finney, 1971).

RESULTS

Effect of L-NAME on forced swim test in mice: The L-NAME at dose of 5, 10 and 20 mg kg^{-1} produced a decrease in immobility duration (sec) with respect to vehicle control group ($p<0.01$) as shown in Fig. 1. This reduction in the immobility duration was dose dependent from 5 to 10 mg kg^{-1} dose but however, it was not found to be dose dependent from 10 to 20 mg kg^{-1} dose. The L-NAME at dose of 2.5 mg kg^{-1} did not alter the immobility duration of the mice. Imipramine used as positive control showed significant decreased in immobility duration ($p<0.01$). The L-NAME at dose of 5, 10 and 20 mg kg^{-1} produced 43.86, 64.33 and 57.08% reduction in immobility duration, respectively in the forced swim test compared with vehicle control group. The percent reduction in immobility duration for imipramine (10 mg kg^{-1}) was 66.38%. The ED_{50} of L-NAME is 9.05 mg kg^{-1} (lower limit 4.328, upper limit 18.922), calculated by Finneys probit analysis method.

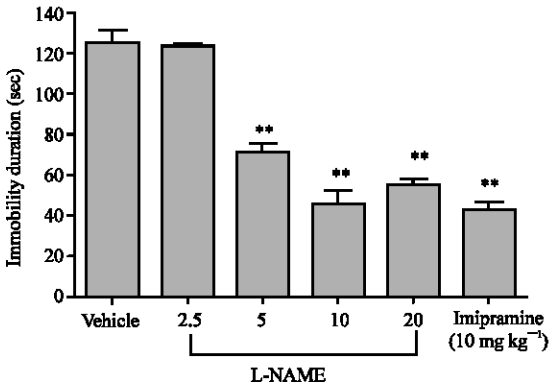

Fig. 1: Effect of L-NAME (2.5, 5, 10 and 20 mg kg^{-1}) and imipramine (20 mg kg^{-1}) on the mean immobility duration in mice forced swim test. The values are expressed as Mean±SEM. Data were analyzed by one way Analysis of Variances (ANOVA) followed by Bonferroni test. **$p<0.01$ compared with vehicle control group. ED_{50} = 9.049

Interaction of L-NAME with agonist and or antagonist of different receptors

Effect of pretreatment of clonidine with antidepressant like action of L-NAME in forced swim test: As shown in Fig. 2, clonidine (150 μg kg^{-1}) significantly potentiates the immobility duration of mice as compared to vehicle control group ($p< 0.01$). This increase in immobility duration was significantly reversed by L-NAME (10 mg kg^{-1}) in clonidine pretreated animals ($p<0.01$). Increase in percent immobility duration of clonidine was found to be 23% against vehicle treated group whereas, reduction in percent immobility duration by L-NAME in clonidine pretreated group was found to be 53.54% against clonidine alone treated group.

Effect of pretreatment of reserpine with antidepressant like action of L-NAME in forced swim test: Reserpine (2 mg kg^{-1}) administered before 4 h significantly increased the immobility duration of mice ($p<0.01$) in forced swim test as shown in Fig. 3. The L-NAME (10 mg kg^{-1}) when administered after 3 h in reserpinized mice significantly

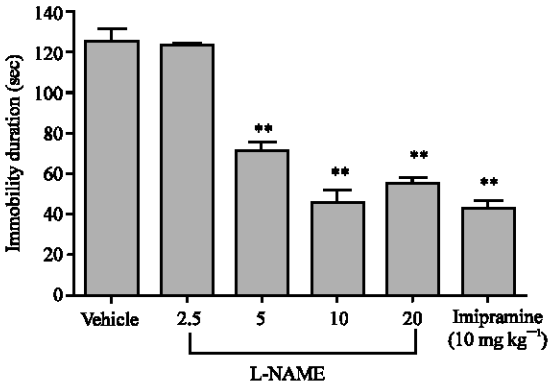

Fig. 2: Effect of L-NAME on clonidine-induced depression in forced swim test. Clonidine (150 μg kg^{-1}) administered 15 min before the treatment of L-NAME (10 mg kg^{-1}, i.p.). The values are expressed as Mean±SEM. Data were analyzed by one way Analysis of Variances (ANOVA) followed by Bonferroni test. **p<0.01 compared with vehicle control group

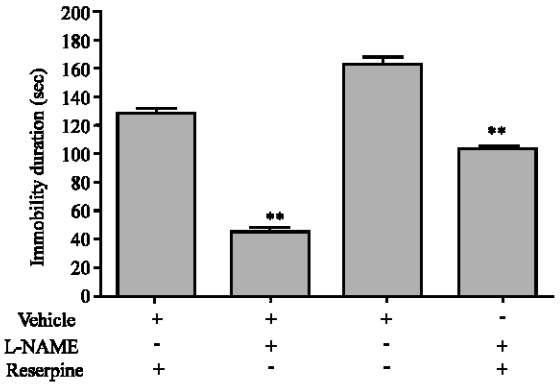

Fig. 3: Effect of L-NAME on reserpine-induced depression in forced swim test. The values are expressed as Mean±SEM. Data were analyzed by one way Analysis of Variances (ANOVA) followed by Bonferroni test. **p<0.01 compared with vehicle control group

reversed immobility duration (p<0.01). Percent increase in immobility duration by reserpine was found to be 19% as compared to that of vehicle treated animals whereas L-NAME (10 mg kg^{-1}) reduced the percent immobility duration to 36.99% compared with reserpine treated animals.

Effect of pretreatment of haloperidol with antidepressant like action of L-NAME in forced swim test: Haloperidol (2 mg kg^{-1}) did not alter the immobility duration of mice in forced swim test as shown in Fig. 4. However, L-NAME

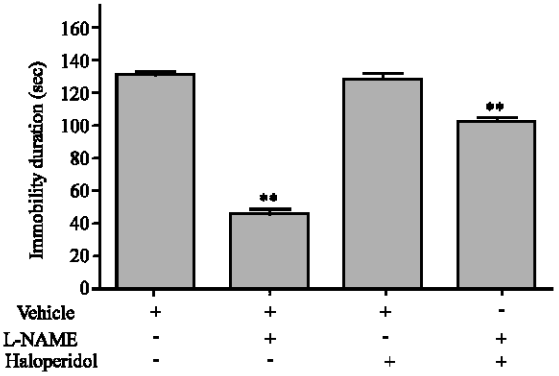

Fig. 4: Effect of L-NAME on haloperidol treated group in forced swim test. Haloperidol (2 mg kg^{-1}) was found to be ineffective in reducing immobility duration. The values are expressed as Mean±SEM. Data were analyzed by one way Analysis of Variances (ANOVA) followed by Bonferroni test. **p<0.01 compared with vehicle control group

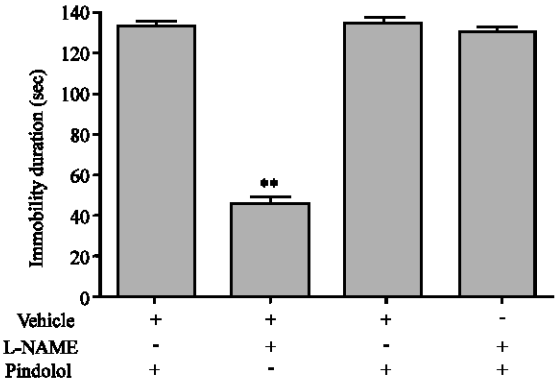

Fig. 5: Effect of pretreatment of mice with pindolol (10 mg kg^{-1}, 5-HT1A/1B receptor antagonist) on the L-NAME-elicited decrease in the immobility time in forced swim test. The values are expressed as Mean±SEM. Data were analyzed by one way Analysis of Variances (ANOVA) followed by Bonferroni test. **p<0.01 compared with vehicle control group

administered in haloperidol pretreated animal partially reduced the immobility duration in mice compared to haloperidol per se treated animal. The (p<0.01) percent reduction in immobility duration by L-NAME in haloperidol pretreated animal was found to be 21.14% as compared with haloperidol treated animal.

Effect of pretreatment of pindolol with antidepressant like action of L-NAME in forced swim test: Figure 5 shows

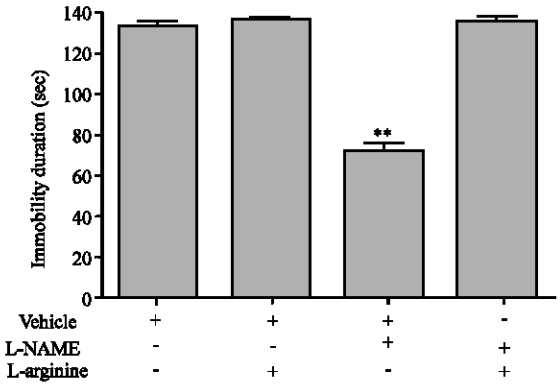

Fig. 6: Effect of L-NAME on nitric oxide modulators like L-arginine on mice in forced swim test. The values are expressed as Mean±SEM. Data were analyzed by one way Analysis of Variances (ANOVA) followed by Bonferroni test. **p<0.01 compared with vehicle control group

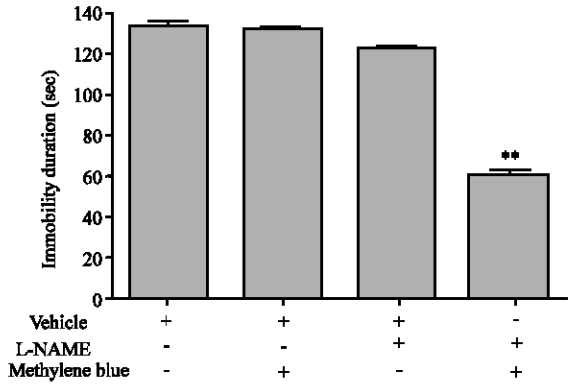

Fig. 7: Effect of L-NAME on nitric oxide modulators like methylene blue (10 mg kg^{-1}) on mice in forced swim test. The values are expressed as Mean±SEM. Data were analyzed by one way Analysis of Variances (ANOVA) followed by Bonferroni test. **p<0.01 compared with vehicle control group

that pretreatment of mice with pindolol (10 mg kg^{-1}) antagonized the anti-immobility effect of L-NAME (10 mg kg^{-1}) in the forced swim test. Also, reversal of the antidepressant like effect of L-NAME by pindolol. Percent reduction in immobility duration of L-NAME in pindolol treated group as compared to that of vehicle control group was found to be 2.48%, respectively.

Effect of pretreatment of various nitric oxide modulators on the antidepressant activity of l-NAME

Effect of pretreatment of L-arginine with antidepressant like action of L-NAME in forced swim test: Figure 6 shows that pretreatment with sub effective dose of L-arginine (750 mg kg^{-1} nitric oxide precursor) did not have any effect on immobility duration of mice(p>0.05). The L-arginine reversed the antidepressant action of L-NAME by an increasing in immobility duration in forced swim test compared to L-NAME (5 mg kg^{-1}) per se group. Percent immobility reduction for L-NAME in L-arginine animals was found to be -0.75% compared to its vehicle control group.

Effect of pretreatment of Methylene blue with antidepressant like action of L-NAME in forced swim test: Methylene blue per se did not alter the immobility duration of the mice (Fig. 7). When L-NAME (2.5 mg kg^{-1}) dose was co-administered with methylene blue, it significantly reduced the immobility duration compared to vehicle, L-NAME and methylene blue treated animals (p<0.01). Percent immobility reduction for L-NAME in methylene blue treated animals was found to be 54.0% compared to its vehicle control group.

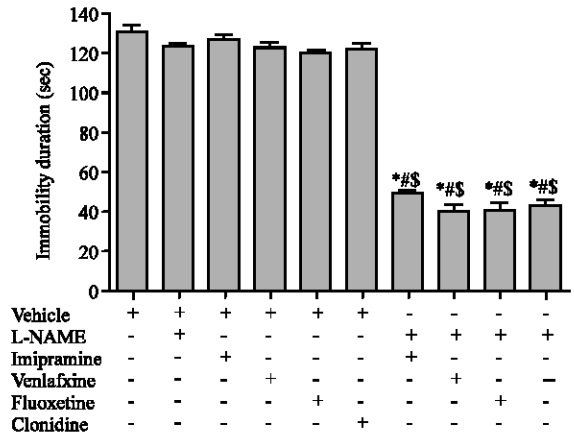

Fig. 8: Effect of L-NAME on, imipramine, venlafaxine, fluoxetine and clorgyline induced immobility time in the forced swim test. Mice were simultaneously treated with L-NAME and standard antidepressants. After one 1 h test were carried out. The values are expressed as Mean±SEM. Data were analyzed by one way Analysis of Variance (ANOVA) followed by Bonferroni test. *p<0.01 with compared with vehicle treated animal, #p<0.01 with compared to L-NAME alone treated animals, $p<0.01 with compared to its respective standard antidepressant treated animals

Effect of L-NAME with various standard antidepressants on immobility periods in forced swim test: The results shown in Fig. 8 indicated the effect of pretreatment of mice with imipramine (2.5 mg kg^{-1}),

Table 1: Effect of L-NAME on locomotor activity in mice

Treatments	Dose	Mean count: Ambulations	Mean count: Stereotypes
Vehicle	-	901.8±8.59	840.0±11.3
	5 mg kg^{-1}	914.3±7.34	905.6 ± 5.1
L-NAME	10 mg kg^{-1}	891.5±11.2	844.0±11.3
	20 mg kg^{-1}	828.1±10.4	864.5±6.9
Chlorpromazine	10 mg kg^{-1}	183.6±9.12***	176.5±4.4***

Values are Mean±SEM. ***p<0.001

venlafaxine (2 mg kg^{-1}), fluoxetine (5 mg kg^{-1}) and clorgyline (0.125 mg kg^{-1}), respectively, on the reduction in immobility duration elicited by L-NAME (2.5 mg kg^{-1}). Pretreatment with Imipramine, venlafaxine, fluoxetine and clorgyline augmented the antidepressant-like activity of L-NAME in mouse forced swim test. The percent reduction in immobility duration was 54.94, 67.13, 66.29 and 64.80% of L-NAME in imipramine, venlafaxine, fluoxetine and clonidine treated group, respectively.

Effect of L-NAME on locomotor activity in forced swim test: Table 1 shows that L-NAME at dose of 5, 10 and 20 mg kg^{-1} i.p., did not produce any effect on the ambulatory and stereotypic locomotor activity of Swiss mice ($p>0.05$). However reference positive standard chlorpromazine (10 mg kg^{-1} p.o.) significantly reduced the both ambulatory and stereotypic movements in the mice ($p<0.001$).

DISCUSSION

The present study demonstrated antidepressant like action of L-NAME in the FST. The L-NAME reduced the immobility duration in Swiss mice. However, this reduction of immobility duration was not found to be dose dependent. This study was carried out with low but effective dose of L-NAME. Further an attempt was made to explore the possible mechanism of action of L-NAME by studying its interaction with different agonist and or antagonist of different receptors, NO modulators and with standard antidepressant drugs.

The FST, also known as behavior despair test is world-widely used as reliable animal model of depression to screen new antidepressant as well as to investigate the mechanisms underlying the action of antidepressants. (Wang *et al*., 2007). 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. The FST of depression is based on the observation that rats or mice when forced to swim or suspended in a restricted space from which there is no possibility of an escape, eventually cease to struggle, surrendering themselves to the experimental conditions. This suggested by Porsolt *et al*. (1978) that this helplessness or despair behavior reflected a state of lowered mood in laboratory animals and could serve as a valuable test for screening antidepressant drugs.

In the present study, intraperitonial administration of L-NAME induced antidepressant-like effect in mice. We observed saturation in the reduction of immobility duration between 10 and 20 mg kg^{-1} dose. In this study, we got reduction in immobility duration with very low doses of L-NAME. Percent reduction in the immobility duration of 10 mg kg^{-1} L-NAME dose was comparable with that of reference positive standard imipramine (10 mg kg^{-1} i.p.). The L-NAME at the tested doses did not affect the ambulatory or stereotypic locomotor activity of the Swiss mice.

Earlier studies have suggested that NO may be able to stimulate the release of neurotransmitters such as dopamine and norepinehrine (Zhu and Luo, 1992; Strasser *et al*., 1994). The NO plays the role of an inhibitory endogenous substance in discriminative effects of psychostimulants in rats, because inhibition of NO synthase enhances the effects of amphetamine and cocaine while an increase NO attenuates them (Filip and Przegalinski, 1998).

It is well known that depressive state is mainly produced by deficiency of norepinephrine, serotonin and dopamine in the brain. The therapeutic effects of antidepressants are believed to be related to their effects on neurotransmitters. Either they block the breakdown of monoamine neurotransmitters such as norepinephrine and serotonin (MAOIs) or prevent reuptake of various neurotransmitters including serotonin, norepinephrine and dopamine (TCAs, SSRI and SNRI). To explore the involvement of norepinephrine, serotonin and dopamine in antidepressant like action of L-NAME in FST, interaction of L-NAME with agonist and or antagonist of receptors involved in depression were studied.

Clonidine an $alpha_2$ adrenoceptor agonist, which depletes catecholamine or lower the noradrenergic turn over in the brain produced depression-like syndrome in animals. This characteristic effect of clonidine was reversed by NOS inhibitor, L-NAME. The underline mechanism for reversal of clonidine-induced behavioral despair by L-NAME may be facilitation of noradrenergic activity resulting from inhibition of norepinephrine uptake, interaction with presynaptic alpha adrenoceptor or MAO inhibition. This observation suggests that adrenergic receptor may be involved in the antidepressant like action of L-NAME.

Reserpine, a vesicular re-uptake blocker, which depletes catecholamine or lowers noradrenaline turnover

in the brain produced depression like syndrome in animals (Tripathi, 2004). Since, reserpine induced depressive state was significantly reversed by L-NAME, tempting to suggest the biogenic amines are involved in antidepressant action of L-NAME. In the present study, pre-treatment with haloperidol, a dopamine (D_2) receptor agonist, partially reduced the immobility duration of L-NAME, suggests that dopamine receptor may be involved in the antidepressant like action of L-NAME.

It is known that 5-HT_{1A} receptor plays a major role in pathogenesis of depression as well as the antidepressant response. The 5-HT_{1A} receptor may be related to the antidepressant responses of selective serotonin reuptake inhibitor in behavioral model of depression. In order to study the possible involvement of 5-HT_{1A} receptor in the antidepressant like effect of L-NAME, pindolol, a 5- $HT_{1A/1B}$ receptor antagonist was used in the present study. As a 5-$HT_{1A/1B}$ receptor antagonist, pindolol fully blocked the antidepressant like effect of L-NAME in this study. Although, pindolol is also known to block the β adrenoceptor, it is unlikely that this property is related to the effect of L-NAME, as β adrenoceptor inhibitors has been reported to increase the incidences of depression. It suggests that 5-HT_{1A} receptor may be involved in the antidepressant like action of L-NAME.

In other set of experiments, we explored the possible involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant like action of L-NAME. The Nitric Oxide (NO) cyclic guanosine monophosphate (cGMP) is an important signaling pathway that has been recently implicated in depression. In conjunction with our evidence that NO synthase inhibitors have antidepressant activity, a potential role for NO in affective disorders has also recently been proposed. The NO is a regulator of both short- and long-term neuronal adaptive changes and consequently, may play a role in neuronal adaptation to antidepressant drugs. The NO is proposed to modulate synaptic transmission in several ways, the most common being through activation of soluble guanylate cyclase and nitrosilation of protein and enzymes (Snyder and Ferris 2000; Prast and Philippu, 2001). The L-arginine is reported to exert a U-shape effect in the FST, doses ranging from 30, 100 to 1000 mg kg^{-1} with lower dose causing no alteration, middle dose causing statistically significant reduction and higher doses causing no alteration in the immobility time, respectively (Ergun and Ergun, 2007). We have chosen the dose of L-arginine (750 mg kg^{-1} i.p.) according to the literature available (Dhir and Kulkarni, 2007; Kaster *et al*., 2005). This dose did not affect the immobility period. In the present study, pretreatment with L-arginine reversed the antidepressant action of L-NAME (5 mg kg^{-1} i.p.). This suggests that NO signaling pathway is involved in the antidepressant like action of L-NAME. Present finding is in agreement with Jefferys and Funder (1996), who also reported reversal of antidepressant action of L-NAME in FST by L-arginine. It is believed that these NO-mediated behavioral changes could be due to, at least in part, soluble guanylate cyclase activation, because the inhibition of these also induced antidepressant like effect in rodents (Eroglu and Caglayan, 1997; Heiberg *et al*., 2002) and humans. Methylene blue, a direct inhibitor of both NOS and soluble guanylate cyclase was used in this study. Methylene blue (10 mg kg^{-1} i.p.) was used at its sub therapeutic dose, did not produce any effect on immobility duration of the mice. When L-NAME (2.5 mg kg^{-1} i.p.) co-administered with methylene blue to the mice in the FST, significantly reduced the immobility duration in the mice. This finding indicates that soluble guanylate cyclase is involved in the antidepressant like action of L-NAME. Thus, these results indicated that the inhibition of NO synthesis may underlie the reduction in the immobility period in the FST elicited by L-NAME.

Earlier studies have suggested that 7-nitroindazole (50 mg kg^{-1}), a specific neuronal NOS inhibitor of both NOS and sGC (Patil *et al*., 2005), inhibit the immobility time in FST. The NO's potential role in the CNS became increasingly clear following reports that NOS inhibitors (e.g., N-methyl-arginine) blocked the pronounced elevations of cyclic GMP that often resulted from glutamate stimulation of the NMDA receptor.

In another set of experiment we explored the interaction of L-NAME with standard antidepressant drugs. Traditional antidepressant, such as tricyclic antidepressants (TCA), Selective Serotonin Reuptake Inhibitors (SSRI), Serotonin and Noradrenaline Reuptake Inhibitors (SNRI) and Monoamine Oxidase Inhibitors (MAOI), increased the concentration of noradrenaline, serotonin and dopamine by either inhibiting neurotransmitter reuptake or its degradation.

Fluoxetine is a potent inhibitor of serotonin reuptake with least or no effect on norepinephrine reuptake (Mochizucki, 2004). Unlike these agents, imipramine or venlafaxine are dual reuptake inhibitor of serotonin and norepinephrine (Mochizucki, 2004; Sindrup *et al*., 2003). Low dose of venlafaxine inhibited serotonin reuptake while at high dose it inhibited both serotonin and norepinephrine reuptake and has an action somewhat similar to Imipramine (Dhir and Kulkarni, 2007). In the present study we observed that L-NAME, when administered with standard antidepressant drugs such as imipramine, venlafaxine, fluxetine and chlorgylline at sub therapeutic dose produced synergistic action in

FST. The NOS inhibitor increased extracellular levels of serotonin and dopamine in the rat ventral hippocampus after local or systemic administration (Wegener *et al*., 2000). Similarly report has shown that NOS inhibitor, N(G)-nitro-L-arginine (L-NA) augmented the behavioral effect of imipramine or fluxetine in FST (Harkin *et al*., 2004). These studies argue for the possibility of inhibition of NOS could be a strategy to enhance the clinical efficacy of various antidepressants.

CONCLUSION

The results of the present study conclude that L-NAME possessed antidepressant like activity in mouse model of behavior despair. It is possibly by modulation of various biogenic amines and L-arginin-nitric oxide cyclic guanosine monophosphate pathway.

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