

**TO EVALUATE THE ANTI- DEPRESSANT EFFECT OF ASCORBIC ACID ALONE AND AS AN ADJUVANT WITH VENLAFAXINE IN MICE*****Sarita Goyal and MC Gupta**

Pharmacology Department, Pt. B D Sharma Post Graduate Institute of Medical Sciences, Rohtak (Haryana).

***Corresponding Author: Dr. Sarita Goyal**

Pharmacology Department, Pt. B D Sharma Post Graduate Institute of Medical Sciences, Rohtak (Haryana).

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ABSTRACT

Ascorbic acid, commonly known as vitamin C, is a water-soluble antioxidant vitamin and has several therapeutic applications in a wide range of diseases. It is considered as a neuromodulator as it is highly concentrated in the brain and, as such, has a possible therapeutic relevance for the treatment of depression. This study was conducted to investigate the anti-depressant effect of ascorbic acid alone and as an adjuvant with venlafaxine in forced swimming test (FST) and tail suspension test (TST) in mice. 24 male mice were selected and divided into 4 groups. Group I received normal saline. Group II received venlafaxine (48mg/Kg); Group III received ascorbic acid 130mg/Kg and Group IV received both Venlafaxine + ascorbic acid (48mg/Kg + 130 mg/Kg. All animals received drug treatment per oral (p.o.), one hour before the test. The results of the study showed that ascorbic acid produces an antidepressant like effect in TST and FST. In addition it potentiates the action of the conventional antidepressant venlafaxine, when given in combination.

KEYWORDS: Ascorbic acid, forced swimming test (FST), tail suspension test (TST).**INTRODUCTION**

Depression can be a long lasting or recurrent illness which can impair an individual's ability to work or cope with daily life.^[1] At a global level, over 300 million people are estimated to suffer from depression, equivalent to 4.4% of the world's population.^[1] Depression is a common and highly heterogeneous psychiatric disorder and it is the third leading cause of global disease burden, in terms of public health significance.^[2] The illness can be attributed to a wide variety of abnormal variations in an individual's mood, which is characterized by periods of depressed mood, extreme sadness, or loss of interest in activities, insomnia or increased sleep etc. Studies have shown that there is a strong association between depression and excess mortality causing high impact on public health.^[3] Association of depression and suicide is well established and studies have shown that the probability of deaths among depressed hospitalized patients is around 15%.^[4]

Most commonly used medications, to treat depression, often referred to as second-generation antidepressants, are selective serotonin reuptake inhibitors (e.g. sertraline, fluoxetine), serotonergic noradrenergic reuptake inhibitors (duloxetine, desvenlafaxine), which have less toxicity and improved safety compared to the first-generation drugs, which include monoamine oxidase inhibitors (e.g. moclobemide), tricyclic antidepressants (e.g. amitriptyline), and atypical antidepressants (e.g. venlafaxine).^[5] The main drawback of the currently

available treatments remain sub-optimal, with a delay of 3–6 weeks before their clinical effects can be achieved and a lack of efficacy is also observed in many cases.^[6] Many a time, patients of depression do not respond to standard treatment and then adjuvant therapy is indicated. For these reasons, the discovery of new drugs or innovative compounds that could further improve current depression therapies are welcomed.^[7]

Ascorbic acid is one of the important water soluble vitamins; humans cannot synthesize ascorbic acid due to the absence of the enzyme L-gulonolactone oxidase. It has many physiological functions and an antioxidant effect and acts as a cofactor in the synthesis of neurotransmitters.^[8] Recent evidence suggests that oxidative stress processes might play a relevant role in the pathogenic mechanism(s) underlying many major psychiatric disorders, including depression.⁹ Reactive oxygen has been shown to modulate levels and activity of norepinephrine (NE), serotonin (5HT), dopamine (DA) and glutamate; the principal neurotransmitters involved in the neurobiology of depression.^[9] Moderately low levels of ascorbic acid have been linked to depression. New potential targets for the development of therapeutic interventions are based on antioxidant compounds.^[10] In clinical studies, it has been reported that the administration of ascorbic acid relieved ACTH-induced depression in a child^[11] and decreased scores in a Beck Depression Inventory in healthy young adults, indicating mood improvement.^[12] Moreover, a recent

study carried by us demonstrated that administration of ascorbic acid in mice through intraperitoneal (i.p.) injection produced an antidepressant-like effect at different doses, in the tail suspension test (TST), and in the forced swimming test (FST), two widely used behavioral tests to predict the efficacy of antidepressant treatments.^[13]

Given the fact that our results suggest that ascorbic acid produces antidepressant actions, the aim of the present study was to investigate the possible antidepressant-like effect of ascorbic acid administered by (p.o.) and in addition, its effect in combination with conventional antidepressants venlafaxine.

MATERIALS AND METHODS

Animals

The behavioral experiments were conducted by using adult Swiss mice of either sex, weighing 20–40 g, which were maintained at 22–25 °C with free access to water and food, under a 12/12 h light–dark cycle, with lights on at 07:00 am. All manipulations were carried out between 09:00 and 16:00 hours, with each animal being used only once. All procedures in this study were performed in accordance with the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India and were approved from the Institutional Ethics Committee.

Grouping of Animals

Twenty-four animals were divided into four groups of six each. Group I served as control and received comparable amount of 0.9% saline only. Group II served as standard and received 48 mg/kg venlafaxine. Group III received 130 mg/kg of ascorbic acid and Group IV received 48 mg/kg of venlafaxine and 130 mg/kg of ascorbic acid, respectively. All the animal groups received single dose of the above-mentioned drugs by oral route (p.o.), 1 hour before the test.

Antidepressant activity

Two accepted and commonly used models of depression for the present study were included.

1. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru *et al.* (1985).^[14] Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The mice were considered immobile only when they were rendered completely motionless on being hung passively. Immobility time was manually recorded during a 6-min period. Readings were taken at baseline and again after one hour of giving drugs.

2. Forced swim test (FST)

The FST was performed as described previously by Porsolt *et al.*^[15] The test was carried out in two sessions.

In the first session i.e in training session, individual mice were forced to swim in an open cylindrical container having 25 cm height, 10 cm diameter and containing depth of water 15 cm at temperature $25 \pm 1^\circ\text{C}$. After 24 h of training session, the FST was performed for 6 min and following parameters were noted i.e.

Immobility: when mice stopped struggling, remained floating motionless in the water and do only necessary movements to keep their head above water, indicative of a depressant-like effect;

Climbing: vigorous movements usually directed against the wall of the tank with the forepaws in and out of water and.

Swimming: large forepaw movements that displaced water to move the body around the cylinder, more than necessary to keep the head above water. The water was changed after every session to avoid the influence of water temperature and urinary or faecal material.

STATISTICAL ANALYSIS

All the data represented mean \pm SEM and was analysed by means of one-way analysis of Variance followed by Dunnett's test. The *p* value <0.05 was considered significant.

RESULTS

Effect of ascorbic acid alone and as an adjuvant in FST

The results depicted in (Figure 1) show that the administration of ascorbic acid by p.o. route decreased the immobility time and increased swimming as well as climbing time as compared with normal saline group, indicating that the systemic administration of ascorbic acid is effective in producing an antidepressant like effect. Administration of ascorbic acid with conventional venlafaxine decreased the immobility time significantly ($p < 0.01$).

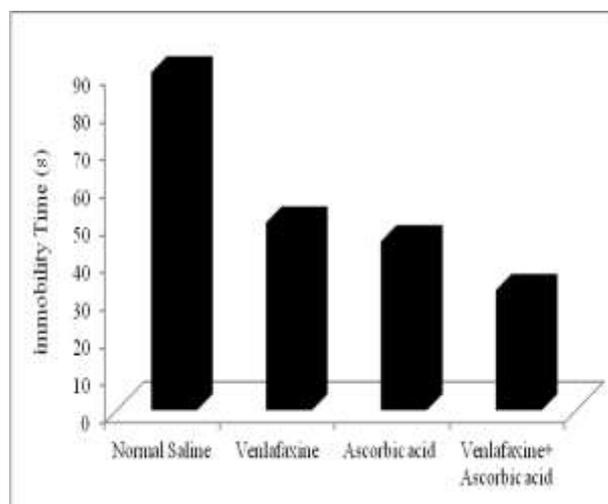


Figure 1(A).

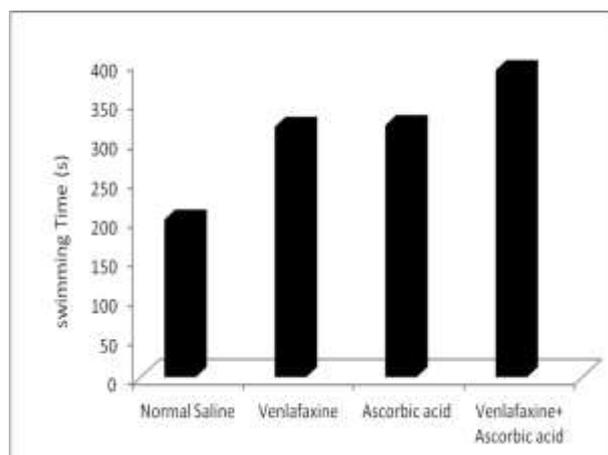


Figure 1(B).

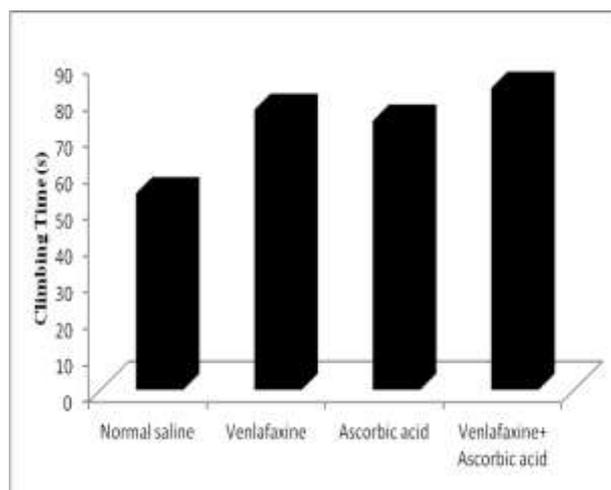


Figure 1(C).

Effect ascorbic acid alone and as an adjuvant in TST

In TST, ascorbic acid alone as well as an adjuvant with venlafaxine showed a significant ($p < 0.01$) decrease in the period of immobility (Figure 2) and increase in the escape time as compared with normal saline group and it was comparable with venlafaxine group, implying that its anti-depressant activity was similar that of venlafaxine.

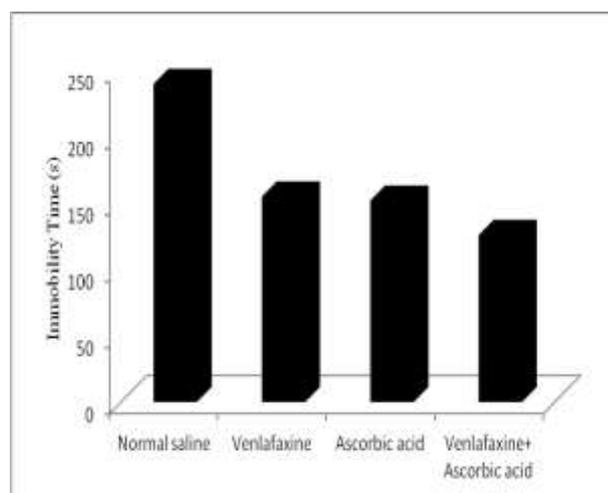


Figure 2.

DISCUSSION

The present study was aimed to evaluate the antidepressant effect of ascorbic acid alone as well as an adjuvant medication with venlafaxine, with the help of two most widely used animal models for antidepressant screening that is FST and TSTs.

A substantial number of studies have reported that mice or rats exposed to chronic stress exhibit depressive-like behavior, evidenced by increased immobility period in behavioral despair tests, particularly in the TST and FST.^[16, 17]

The results of the present study showed that ascorbic acid given systemically by p.o. route produces an antidepressant like effect in TST and FST, which is dependent on an interaction with the monoaminergic system. In addition, ascorbic acid was able to potentiate the action of conventional antidepressant venlafaxine in this predictive test of antidepressant action, reinforcing the idea that this vitamin has antidepressant properties.

Antidepressant-like effect of ascorbic acid may be dependent on different properties of this vitamin, such as its neuromodulatory and antioxidant actions. Various studies have shown that in the central nervous system, particularly in neurons, ascorbic acid is maintained at elevated concentrations and may act as a neuromodulator, facilitating the release of some neurotransmitters and inhibiting neurotransmitter binding to receptors, including responses mediated by glutamatergic system,^[18,19,20] which is proposed to represent a key role in the pathophysiology of depression.^[21] In the same scenario, ascorbate transport to the extracellular milieu has been linked to glutamate uptake in a process termed ‘‘ascorbate-glutamate heteroexchange’’,^[22] which decreases extracellular glutamate levels and, in turn, reduces excitotoxicity and pro-oxidative damage. Recently, it has been shown that the mechanism by which ascorbic acid exerts its anti-immobility effect in mice is dependent, at least in part, on the NMDA receptor inhibition.^[23] Consistent with this notion, previous results provided direct evidence that NMDA receptor antagonists, such as ketamine and Ro 25-6981 (a selective NR2B antagonist), rapidly reverse the behavioral, morphological and physiological deficits resulting from chronic stress.^[24,25]

On the basis of present study, we conclude that ascorbic acid potentiates the effect of conventional antidepressants such as venlafaxine in the TST and FST, without modifying the ambulatory behavior. In several cases these conventional antidepressants are ineffective, poorly tolerable and present a long delay to achieve their therapeutic effects.^[6] Considering the need for faster acting, safer and more effective treatments for depression, the synergistic antidepressant-like effect between ascorbic acid and the prescribed antidepressants suggests that this vitamin could be helpful for the improvement of the conventional pharmacotherapy

(decreasing the doses of antidepressants prescribed and consequently the side effects). Long-term and larger prospective studies need to be conducted to validate the anti-depressant role of ascorbic acid so that it could constitute a new and attractive strategy for the treatment of depression.

CONCLUSION

The interesting finding of this study is that ascorbic acid potentiates the effect of sub-effective dose of venlafaxine when given in combination in TST and FST.

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