A preliminary study on antidepressant activity of NR-ANX-C (a polyherbal product) in mice

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ABSTRACT

Depression is one of the common psychiatric disorders. Though the modern medicine provides large number of antidepressants, each of them has one or the other draw backs. Thus the search for new antidepressants with minimum or without side effects is deemed important. NR-ANX-C, a polyherbal product composed of Withania somnifera, Ocimum sanctum, Camellia sinensis, Triphala and Shilajit have been shown to have antistressor, antianxiety and anticaltaleptic activity. With this back ground, the present study was undertaken to evaluate the antidepressant activity of this polyherbal preparation by employing two experimental models, forced swim test and tail suspension test in mice. Imipramine (5.0 & 10.0 mg/kg) was used as the standard drug. NR-ANX-C (5, 10, & 20 mg/kg) was given orally. Antidepressant effect was studied both on acute and chronic administration. NR-ANX-C significantly reduced the duration of immobility on single and repeated administration. These findings were comparable to that produced by standard drug, imipramine. Our study suggests that, NR-ANX-C has antidepressant like activity in mice.

Keywords: NR-ANX-C, Antidepressant, Imipramine.

INTRODUCTION

Mental depression is one of the common chronic illnesses that affect the mood, thought, physical health and behaviour of an individual. In the recent years it was recognized as a major health problem. Although the mechanism provoking depression has not been clearly elucidated, the main trigger is known to be exposure to chronic stress. In modern medicine several types of synthetic antidepressant drugs are available to treat depression. However, these drugs have the disadvantages of slow onset of action, relatively low response rates and high side effects. Thus, the search for a quick acting new antidepressant drug without or with minimum side effects is need of the hour and it is continuing.

In Ayurveda, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs. NR-ANX-C is a polyherbal product containing aqueous extracts of Withania somnifera, Shilajit, Triphala and the alcoholic extracts of Camellia sinensis and Ocimum sanctum. These constituents are being used as nerve tonic and antistressor agents and found to have effects on monoaminergic and gama amino butyric acid (GABA) ergic systems along with antioxidant properties. From our laboratory, we have reported the antianxiety and anticaltaleptic activities of NR-ANX-C. These constituents of the polyherbal products have shown to alter the brain biogenic amines, and have antioxidant properties that prompted us to study antidepressant activity by employing two validated experimental models; forced swim test (FST) and tail suspension test (TST) in mice.

MATERIAL AND METHODS

Animals

Experiments were performed on Swiss male albino mice (25–30g). Animals were procured from the central animal house of the Kasturba Medical College, Mangalore and maintained on a natural day–night cycle (12hr dark: 12hrs light) at room temperature of about 24-26°C, with free access to standard food pellets and water ad libitum. Animals were acclimatized for at least ten days before exposure to behavioral experiments. Experiments were carried out between 09 00 and 14 00 hours by an observer blind to the treatment. The experimental protocol was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Mangalore. Animals were divided into six groups for each experimental method separately for both acute and chronic study and each group consisted of six animals.

Drugs and Dosage

Preparation of drugs

The test drug NR-ANX-C (Standardized and provided by M/s. Natural Remedies Pvt. Ltd. Bangalore) and the standard drug, Imipramine (Ranbaxy, India) were prepared as suspension in 1% gum acacia solution.
Acute Study

Imipramine (5.0 and 10.0 mg/kg) and NR-ANX-C (5, 10 & 20 mg/kg) were given orally single dose, 60 minutes prior to the experiment.

Chronic Study:

Imipramine (5.0 and 10.0 mg/kg) and NR-ANX-C (5, 10 & 20 mg/kg) were administered once a day for 10 days. The last dose was given 60 minutes prior to the exposure to the tests.

Experimental models

A. Forced swim test

The experiment was carried out according to the method described by Porsolt et al.©. Animals were forced to swim individually in glass jar (25 cm height x 12 cm diameter) containing fresh water of 15 cm height and maintained at 23°C ± 2°C for six minutes. Animal was considered to be immobile when it remained floating in water without struggling and making only minimum movements in the limbs necessary to keep the head above the water. Because little immobility is observed during the first two minutes, only that occurring during the last four minutes of the observed six minutes was counted. Each animal was used only once.

B. Tail suspension test

The test was carried out according to the method described by Steru et al.©. Each animal was suspended on the horizontal rod 50 cms above the surface of a table by adhesive tape placed approximately one cm from the tip of the tail. Immobility was recorded during the six minutes of observations. Mice were considered immobile when they hung passively and were completely motionless.

Statistical analysis

The results were expressed as mean ± SEM and analyzed for statistical significance using one-way ANOVA followed by Dunnet’s test. The results were considered to be statistically significant if P< 0.05.

RESULTS

Forced swim test

Acute study

The test was carried out according to the method described by Porsolt et al.©. Each animal was forced to swim individually in glass jar (25 cm height x 12 cm diameter) containing fresh water of 15 cm height and maintained at 23°C ± 2°C for six minutes. Animal was considered to be immobile when it remained floating in water without struggling and making only minimum movements in the limbs necessary to keep the head above the water. Because little immobility is observed during the first two minutes, only that occurring during the last four minutes of the observed six minutes was counted. Each animal was used only once.

Chronic study

Both the standard drug, imipramine (10.0 mg) and the test drug, NR-ANX-C at higher doses (10 mg/kg & 20 mg/kg) significantly (P<0.01) reduced the duration of immobility when compared to vehicle treated group. Percentage of immobility was comparable to that produced by imipramine (10.0 mg/kg & 10.0 mg/kg) and NR-ANX-C (5, 10 & 20 mg/kg) pretreatment was 1.36, 19.47 and 4.6, 9.65 & 17.39 respectively when compared to control vehicle treated group.

Tail suspension test

Acute study

At all the doses tested (5, 10 & 20 mg/kg) NR-ANX-C significantly (P<0.01) reduced the duration of immobility when compared to vehicle treated control group. The reduction was dose related. The effect NR-ANX-C was comparable to that produced by imipramine (5.0 mg/kg & 10.0 mg/kg) and NR-ANX-C (5, 10 & 20 mg/kg) pretreatment was 18.38, 31.97 and 4.6, 9.65 & 17.39 respectively when compared to control vehicle treated group.

NR-ANX-C significantly (P<0.01) reduced the duration of immobility.
immobility in a dose dependent way at all the doses (5, 10 & 20.0 mg/ kg) tested. The standard drug imipramine too significantly reduced the immobility only at the highest dose(10.0mg/kg). Percentage of immobility reduced with imipramine (5.0 mg/kg & 10.0 mg/kg) and NR-ANX-C (5, 10 & 20 mg/kg) pretreatment was 3.97, 8.03 and 13.85,29.94 & 31.17 respectively when compared to control vehicle treated group.

**DISCUSSION**

Depression is a chronic mental disorder affecting more than 10% of population22. The World Health Organization revealed that depression is the fourth leading cause of disability worldwide23. Stressful life events facilitate the evolution of depressive illness24 as the stress can influence the function of central nervous system by altering a number of neurotransmitters, endocrine and neuroendocrine systems7-10. In animals restraint stress is being used as a model of depression. The two rodent models, forced swim test and tail suspension test, in which the animals are exposed to unavoidable stress that produce behavioural despair which reflect a condition similar to human depression25,26. These models are widely used for screening antidepressants and are sensitive to all major classes of antidepressants. In the present study, NR-ANX-C dose dependently reduced the duration of immobility of mice in both experimental models on acute and chronic administration. In FST the reduction was significant only at higher doses (10 mg/kg and 20mg/kg) irrespective of the duration of treatment. However, in case of TST animal’s immobility was reduced with all the doses (5, 10 and 20 mg/kg) test in acute study, while in chronic study the reduction was significant only at the higher doses (10 mg/kg and 20mg/kg). The reduction in the duration of immobility with NR-ANX-C was comparable to that produced by the standard drug imipramine and it is suggestive of antidepressant like activity.

NR-ANX-C is a polyherbal product containing 17% aqueous extract of *Withania somnifera* (2.1% w/w withanolides), 17% alcoholic extract of leaves of *Ocimum sanctum* (contains 2.9% w/w of ursolic acid), 33% of alcoholic extract of the leaves of *Camellia sinensis* (contains total polyphenols 60.1% w/w), 25% of aqueous extract of Triphala (contains 33.5% w/w tannins) and 8% aqueous extract of Shilajit (contains fulvic acid 52.6% w/w and humic acid 16.7%). The constituents that are present in NR-ANX-C like *Withania somnifera*, *Ocimum sanctum*, *Camellia sinensis*, Triphala and Shilajit have been reported to have antistress and antioxidant properties. In addition, they alter the central biogenic amines level and their receptors. Shah et al27 have reported that the antidepressant activity of *Withania somnifera* involves its interaction with adrenoceptor and alteration in central biogenic amines. Recent evidence suggest that *Withania somnifera*, *Camellia sinensis* and *Ocimum sanctum* have antidepressant activity through involvement in adrenoceptors, inhibition of catechol-o-methyl transferase (COMT) and increased biogenic amine level in the brain7-10.

The doses of NR-ANX-C that we have used in this study do not have any significant effect on the motor activity of animal8. Thus, the reduction in the duration of immobility indicates that, it is due to antidepressant activity of the test compound and it is comparable to that of imipramine. Our study suggests that the polyherbal product NR-ANX-C has antidepressant effect and it is comparable to imipramine. But, at this juncture it is difficult to point out which constituent of the product is responsible for the same. Ayurvedic treatises claim that drugs should not be used alone and should be used in combination. This study paves the way for identifying the possible mechanism and the individual ingredient responsible for the antidepressant activity of NR-ANX-C.

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