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Evaluation of Antidepressant Activity of *Nerium indicum* in Chronic Unpredictable Stress Induced Depression

Maheswari Reddy B.*, Praveena Devi C.H.B., Sushma Taj S.K. and Pavan Raj G.

Department of Pharmacology, Joginapally B.R. Pharmacy College, Yenkapally, Moinabad, Hyderabad, Telangana 500075, India

**Corresponding Author*

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Abstract: The objective of the study was to evaluate the anti-depressant activity of hydro-alcoholic extract of *Nerium indicum* in chronic unpredictable stress induced depression. In the present study rats were subjected to chronic unpredictable stress for 14 days and treated with extract as test drug, Fluoxetine as standard. At the end of treatment rats were screened for behavioral parameters like open field test, despair swim test, plus maze test, and biochemical parameters like blood glucose and cholesterol from serum, reduced glutathione, lipid peroxidation, and Catalase were measured from brain homogenate. With the repeated administration of extract 250 mg/kg as well as 500 mg/kg there was profound effects on behavioral parameters and also a significant increase in the serum glucose, cholesterol, superoxide dismutase, reduced glutathione, Catalase levels and a decrease in the lipid peroxidation. The change observed with the treatment with plant extract was significant when compared with standard drug. The present study suggested that hydro-alcoholic extract of *Nerium indicum* significantly reversed stress induced depressive like behavior, oxidative damage and possessed potential antidepressant effect.

Keywords: *Nerium indicum*, Chronic unpredictable stress, Anti-depressant activity, Serum glucose, Cholesterol, Superoxide dismutase, Reduced glutathione, Catalase, Lipid peroxidation

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Introduction

Depression, a widespread incapacitating psychiatric ailment, imposes a substantial health burden on society. It can be defined as a complex syndrome which is characterized by mood disturbance and a variety of cognitive, psychological disturbances. It is the most common

affective disorder that ranges from very mild to severe (psychosis) accompanied with hallucinations. There are so many factors which will affect a person mood, like biological Factors, Genetic factors (Nemeroff, 2007), Physiological factors, and social factors. At its severe stage, it

may leads to suicide symptoms which include sustained low or depressed, decreased interest or pleasure (Kendler *et al.*, 1999), sleep disturbances decreased or increased, guilt or feeling worthless, energy loss or fatigue, concentration problems or problems with memory, appetite disturbance, weight loss or gain, suicidal ideation (Rang *et al.*, 2012). It is characterized by combination of symptoms that interfere with person's ability to pleasurable activities. Currently, the available antidepressant agents are with unwanted side effects, especially they cause insomnia, anxiety and weight gain. Biochemical factors include decrease in the level of neurotransmitters like nor-epinephrine and serotonin in the brain (Tripathi, 2003; Uriguen *et al.*, 2003). Ayurveda, an alternative system of medicine, practiced widely in India, uses several plants (whole/parts) for treatment of variety of diseases. Charaka states-- "A single drug may have many applications owing to its diverse actions just as a man is able to perform various actions". Many popular Ayurvedic drugs such as Ashwagandha, Bramhi, Guduchi, Katuka, Shatavari (Vogel, 2002), etc. have multifarious properties ascribed to them. Since the depressive disorders are having a huge impact on our lives, it is worth evaluating the alternative forms of medicines which can be used for its treatment. So, in this study an effort was made to investigate the antidepressant effect of *Nerium indicum*.

Nerium indicum (syn. *N. oleander* L. and *N. odorum* Aiton) is one such plant used in Indian and Chinese traditional medicinal system for ages. In ethnomedicine, it is used for the treatment of cardiac illnesses, asthma, corns, cancer, and epilepsy. Detailed phytochemical analysis of different parts of the plant have been performed previously. Various bioactivities including antibacterial (Hussian and Gorski, 2004), antinociceptive, anti-anxiety, neuroprotective, hepatoprotective and antioxidant properties of *N. indicum* have already been established. Therefore, considering its ethnomedicinal use and medicinal properties, we have studied the anti-depressant

activity of hydro-alcoholic extract of *Nerium indicum* leaves (HANI).

Materials and Methods

Plant material and extraction:

The fresh leaves of *Nerium indicum* were collected from local areas of Hyderabad, India and authenticated by Dr. H.Ramakrishna, Department of Botany, Osmania University, Telangana, India.

The leaves were separated from the plant and washed properly with distilled water to remove any dirt. The leaves were then shade dried at room temperature for 2 weeks and grinded to powder. The powdered leaves were subjected to extraction by percolation method with ethanol: water (50:50 v/v) at room temperature. The resultant extract was evaporated to dryness using a rotary evaporator (Buchi Type Rotary Vacuum Evaporator, Axiva, Shanghai, China) followed by lyophilization and stored at 4°C for further use. The extract was previously characterized to contain high phenolic and flavonoid content and demonstrated to possess potent free radical scavenging capacity.

Animals:

An ethical approval of this experimental study was obtained from the Institutional Animal Ethical Committee of Malla Reddy College of Pharmacy, Hyderabad (Reg. No 1217/PO/Re/S/08/CPCSEA). Thirty albino rats with average body weight (150 to 250 g) were utilized in this study. They were procured from Teena Labs, Plot no 41, SV Ccooperative Industrial Estates, Bachupally (V), Quthbullapur. The rats were housed in polypropylene cages and maintained under standard conditions (12h light and dark cycles at 25 ±3°C and 35-60 % humidity). Standard pelletized feed and tap water were provided *ad libitum*.

Experimental Design for anti-depressant activity:

The rats were divided into five groups (n=6). Drugs/vehicle were administered to the animals 60 min prior to study.

Group I: Normal control administered with saline 2 ml/kg orally

Group II: Negative control.

Group III: Received *Nerium indicum* 250 mg/kg orally

Group IV: Received *Nerium indicum* 500 mg/kg orally

Group V: Received Fluoxetine 10 mg/kg i.p.

Screening models for anti-depressant activity:

Chronic Unpredictable Stress Model:

Exposure to chronic stress can affect cognitive process and depression in a complex manner depending up on the intensity and duration of stressor. The possible stressors could be: (i) Cold room isolation, (ii) Forced swim test, (iii) Tail suspension, (iv) Restrained stress, (v) Foot shock, (vi) Social isolation, (vii) Wet bedding, (viii) Over crowding, (ix) Food and water deprivation, (x) Cage rotation, (xi) Exposure to loud noise, and (xii) Lights over night.

Forced Swim Test:

The animals were forced to swim in a glass cylinder measuring 25 cm height and 12 cm diameter containing water at room temperature to a depth of 15 cm. After placing the animal in swim test apparatus initially the animal is very vigorous. After 2-3 min activity begins to subside, after 5-6 min immobility reaches a plateau. The rat was considered immobile when it remained floating in the water without struggling (Immobility time), making only minimum movements of its limbs necessary to keep its head above water. After 6 min rat was taken out, dried with a towel. After drug administration Immobility time of animal get reduced which indicates an effective antidepressant activity.

Open Field Method:

In this test 2 days before the actual test every animal was placed in open field for 5 min/day separately. During experimentation the animal was placed in center of the four corners of the field and the following parameters were noted for 5

min. Preference of the animal into central or peripheral arms, total no of entries in central or peripheral arms, average time spent in central or peripheral arms, rearing, grooming, urination and defecation. Each animal was given a score for total locomotor activity as it was calculated by using above parameters.

Elevated Plus Maze Test:

Elevated plus maze was used to study anxiolytic and antidepressant property. Every animal was placed in the Centre of the maze individually, facing towards open arm (Griebel *et al.*, 1997). The following parameters were noted. First preference of the animal to open or closed arm, no. of entries into open or closed arm and average time spent in each arm. The % of animal preference towards open /closed arm was calculated.

Statistical Analysis:

The results are expressed as mean $\pm$ standard error of mean (n = 6). Statistical analysis was done by one way ANOVA followed by Dunnett's comparison test using Computerized Graph pad prism (version 5.0) software at a level of significance of $P < 0.01$.

Results

Phytochemical Screening of HANI:

Phytochemical evaluation revealed the presence of carbohydrates, flavonoids, glycosides, phyto-sterols, saponins and phenolic compounds in Hydro-alcoholic extract of *Nerium indicum* (HANI).

Acute Toxicity study:

The Acute toxicity study (OECD, 2001) suggested that 4000 mg/kg of the extract was safest and non-toxic dose to the rats as no observable acute toxic effects were produced for 48 h and hence 1/8th and 1/16th doses were selected as High dose and low dose, respectively in the present study according to the OECD (423) guidelines.

Biochemical parameters:

Levels of biochemical parameters like Catalase and GSH are significantly decreased and LPO levels

Table 1: Effect of Hydroalcoholic extract of *Nerium indicum* (HANI) on Antioxidant parameters

S. No.	Treatment	LPO (nmol of MDA/ mg protein)	GSH (nmol/mg protein)	Catalase (U/mg protein)
1.	Control	18 ± 0.9	90.6 ± 1.3	214.8±4.2
2.	Stress	30 ± 3.3	55.03 ± 2.5###	144.3±3.9####
3.	Low dose of HANI 250 mg/kg p.o.	28.6 ± 4.9	68.7 ± 1.5*	162.2±1.2*
4.	High dose of HANI 500 mg/kg p.o.	25.9 ± 2.4	72.6 ± 2.7*	178.6±2.0**
5.	Fluoxetine 10 mg/kg i.p.	21.6 ± 0.6	82.7 ± 2.7**	186.2±3.1***

Table 2: Effect of HANI on Glucose and Cholesterol

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)
Control	100.6±0.66	14.3±0.64
Stress	152.7±0.61####	26.5±0.76####
Low dose of HANI 250 mg/kg	129.4±0.49****	21.5±0.76***
High dose of HANI 500 mg/kg	118.6±0.41****	19.1±0.63****
Fluoxetine 10 mg/kg i.p.	114.4±0.48****	16.0±0.63****

were increased in stress control group (Table 1). However, there was an increase in the levels of GSH and Catalase and a decrease in the levels of LPO in extract treated groups in comparison with the standard drug. From the above results it was believed that HANI is having neuroprotective properties as it restores the depleted antioxidant levels.

Table 2 shows the glucose levels of normal and experimental rats after treatment. In normal control group (group I), serum glucose levels were near 100 mg/dl. Glucose levels in the depression-induced group (group II) were higher than 100 mg/dl. In comparison to normal control rats, oral treatment of Fluoxetine (group V) generates a considerable response in Wistar rats, with a significant drop in glucose level. The glucose levels were dramatically reduced after treatment with a

hydroalcoholic extract of HANI in a dose dependant manner (group III and IV).

The current study examined the estimation of rat serum lipid profiles under various conditions. When compared to normal control rats, the levels of total cholesterol were considerably higher in depressed rats (group II). The levels of Total cholesterol in group III and IV were substantially lower than in group II depressed rats after treatment (Table 2). Total cholesterol levels were lower in rats treated with standard drug Fluoxetine and the levels were similar to normal control rats. Individuals with depression had slightly higher total cholesterol readings (Table 2).

Behavioral parameters:

Table 3 shows the antidepressant effect of HANI and Fluoxetine in the experimental animals. The control animals remained immobile for most of

Table 3: Effect of HANI on Immobility time in Forced Swim test

Treatment	Immobility time (sec)	No. of Counts
Control	116.5 ± 4.7	26 ± 2.3
Stress	178.4 ± 6.4####	13.6 ± 1.5##
Low dose of HANI 250 mg/kg p.o.	168.2 ± 5.1	22.6 ± 1.1*
High dose of HANI 500 mg/kg p.o.	144.6 ± 4**	23.2 ± 1.8*
Fluoxetine 10 mg/kg i.p.	134.3 ± 6***	24.8 ± 2.6**

Table 4: Effect of HANI in Open field test

Treatment	No of Entries	No of Rears
Control	92 ± 1.7	28 ± 3.6
Negative control	64 ± 2.6####	49 ± 4.1###
Low dose of HANI 250 mg/kg p.o.	79 ± 2.1*	38 ± 3.7*
High dose of HANI 500 mg/kg p.o.	82 ± 2.8**	36 ± 2.6*
Fluoxetine 10 mg/kg i.p.	88 ± 2.7***	32 ± 3.3**

Table 5: Effect of HANI on Elevated plus maze

Treatment	Open arm exploration		
	% Preference	No. of entries	Time spent
Control	44 ± 2.9	12 ± 0.7	148.3 ± 3.2
Stress	16 ± 1.3####	3 ± 0.7####	88.0 ± 4.3####
Low dose of HANI 250 mg/kg p.o.	22 ± 3.1*	8 ± 1.4*	118.0 ± 1.6*
High dose of HANI 500 mg/kg p.o.	26 ± 4.3**	10 ± 1.1**	122.0 ± 2.2*
Fluoxetine 10 mg/kg i.p.	32 ± 4.1***	11 ± 1.1**	128.0 ± 2.8**

the time during the test session. HANI (250 and 500 mg/kg, p.o.) induced a dose-dependent significant reduction in the immobility time of rats ($p < 0.01$) as compared to the control group. In the same experimental conditions, the antidepressant activity of the reference drug Fluoxetine (10

mg/kg, i.p.) was clearly evident ($p < 0.01$). The antidepressant effect produced by HANI was comparable to that of Fluoxetine.

The results obtained with this animal model showed that Fluoxetine (10 mg/kg) and HANI at all the doses used, significantly decreased the

number of lines crossed by rats (ambulatory activity). It also showed that the number of entries were less and number of rears were more in negative control and a gradual increase in entries and a decrease in the number of rears was observed in the extract treated group. This may indicate that the plant possesses substances that may have anti-depressant properties.

As shown in Table 5, the control animals showed more preference for the closed (dark) arms and exhibited anxiety-like symptoms characterized by immobility, freezing, and defecation on entering the open arms. As compared to the control group, the HANI-treated (250 and 500 mg/kg p.o.) animals showed significant increase in total number of entries ($p < 0.01$) and the time spent in the open arms. Fluoxetine (10 mg/kg, i.p.) a standard drug, significantly increased the number of entries as well as time spent in the open arms ($p < 0.001$) indicating anxiolytic and anti-depressant activity.

All the above values are expressed as Mean $\pm$ S.E.M, $n=6$, One way ANOVA followed by Dennett's test #### $P < 0.001$ when compared with control group, **** $P < 0.001$ when compared with stress group, *** $P < 0.001$ when compared with Stress group, ** $P < 0.01$ when compared with stress group, * $P < 0.05$ when compared with stress group.

Discussion

In this study the antidepressant activity of hydro-alcoholic extract of *Nerium indicum* was measured using Forced swim test, Open field test and Elevated plus maze test. Behavioral tests were used for evaluating the medicines or herbals for their antidepressant activity. Alteration in the levels of glucose, total cholesterol and changes in the antioxidant parameters were also considered to evaluate antidepressant activity of the extract.

The Glucose and Total Cholesterol was increased in the depressed group and reduced in normal and treated groups. In normal control group serum glucose levels were near 100 mg/dl. Glucose levels were high in the depression-induced group in comparison to normal control

rats, oral treatment of Fluoxetine generates a considerable response in Wistar rats, with a significant drop in glucose level. The glucose levels were dramatically reduced after treatment with a hydro-alcoholic extract of HANI in a dose dependant manner.

Glucose is the primary energy source for brain cells, and the glucose transporter (GLUT) family is responsible for its entry (Vannucci *et al.*, 1997). In the human brain, glucose transporters 1 (GLUT1) and 3 (GLUT3) are primarily transmembrane glucose transporters (Klepper *et al.*, 2007). DNA methylation of the main promoter regions of GLUT1 was much higher in depression patients' brain cells than in healthy comparison subjects, lowering the efficacy of GLUT1 to absorb glucose from blood vessels to cells and compromising brain metabolism. After depression patients were treated, DNA methylation of the GLUT1 promoter was significantly reduced. This could indicate that GLUT1 increases are linked to good depression treatment (Kahl *et al.*, 2016). Li *et al.* (2020) and Linda *et al.* (2011) showed that antidepressant effects are linked to glucose metabolism.

Recently, oxidative stress was linked with the pathophysiology of major depression, with significant correlations being found between the severity of depression and erythrocyte superoxide dismutase/lipoperoxidation levels. Meanwhile, treatment with antidepressants reduces the oxidative stress related to depressive disorder. Additionally, *Nerium indicum* reported to have antidepressant-like properties, also possess antioxidant activity. Therefore, it is possible that the antioxidant activity of the hydroalcoholic extract from *Nerium indicum* may contribute to its antidepressant effect.

In the behavior tests, despair test has been validated as a suitable tool to evaluate drugs with putative antidepressant effects (Porsolt *et al.*, 1978). In this model, when rodents are forced to swim in a confined space, they tend to become immobile after vigorous activity (struggling). This inescapable stressful situation leads to depression. In the present study, administration of HANI

significantly reduced total immobility time and enhanced struggling behavior in a dose-dependent manner, suggesting an antidepressant effect. It is reported that GABA, an inhibitory neurotransmitter is involved in the pathophysiology of depression. Moreover, neurochemical research has revealed that the monoamines (5-HT, NA, and dopamine) have a crucial role in the development of the depression syndrome (Naughton *et al.*, 2000). The antidepressant effect of the HANI may be attributed to the modulation of one or more of these neurotransmitters. It has been found that flavonoids isolated from plant species such as *Nerium indicum* might be responsible for exhibiting antidepressant activity. Thus, it is likely that flavonoids present in HANI may be responsible for the observed antidepressant effect.

The open field test (OFT) was also used for the investigation of the anti-depressant activity. General activity in the OFT measures various behavioural parameters, among those related to emotional, exploratory, and motor behaviours. The first exposure of the animal to the open field has a more marked emotional component than the remaining aspects of exposure (Lazarini *et al.*, 2000). The results obtained with this animal model showed that Fluoxetine (10 mg/kg), HANI at all the doses used, significantly decreased the number of lines crossed by mice (ambulatory activity). Moreover, the time spent at the centre was also slightly increased. This may indicate that the plant possesses substances that may have antidepressant, sedative, or anxiolytic properties.

In the present study, HANI significantly increased number of entries and time spent in the open arms in dose-dependent manner indicating anxiolytic activity. It has been shown that GABAergic neurotransmission plays an important role in stress and anxiety associated with elevated plus maze test (Zwanzger and Rupprecht, 2005). . It is likely that HANI may have modulated the benzodiazepine or other sites of GABA receptors to produce the anxiolytic effect.

Depression is commonly accepted to be a disorder due to disturbances in neurotransmitters

function, particularly serotonin, noradrenaline, and dopamine. Reduction in brain serotonin has been reported to be one of the most important etiological factors for genesis of depression, and the most widely used antidepressants namely SSRIs, increase extracellular availability of serotonin (Schreiber *et al.*, 1995). Thus, HANI may exert its antidepressant effect through one or some of these central nervous neurotransmitters acting on glutaminergic receptors, GABAergic receptor, or serotonergic pathways. At this level, it is not possible to give the exact mechanism of action by which *Nerium indicum* acts. However, we can hypothesize according to our results that the antidepressant effect of HANI appears to be related to enhancement of central noradrenergic and/or serotonergic neurotransmissions.

Phytochemical screening revealed presence of flavonoids, steroids, and β -sitosterol. It can be recalled that several constituents from plants are known to possess neurobehavioral effects in experimental animals. For instance, steroids have been reported as potent sedative agents that inhibited spontaneous motor activity in mice (Dubois *et al.*, 1986). Similarly, flavonoids are reported to possess anxiolytic effect (Wolfman *et al.*, 1994) and antidepressant effect. Therefore, it is likely that flavonoids and steroidal content of this extract might be contributing in part to the observed neuropharmacological activity. The current work demonstrated that acute administration of the HANI produced antidepressant effect. Although the action mechanisms of this extract still to be studied, our results bring the pharmacological evidence of the traditional use of this plant for the treatment of some neurological disorders.

Conclusion

The present results clearly demonstrate that the Hydroalcoholic extract of *Nerium indicum* possesses antidepressant-like activity in the animal behavioral models. The current study warrants further investigation into identification of the active compounds in herbal medicines, in

particular extract of *Nerium indicum* with antidepressant-like effects.

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