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EVALUATION OF ANXIOLYTIC AND ANTIDEPRESSANT POTENTIAL OF HYDRO-ALCOHOLIC LEAVES EXTRACT OF *AZADIRACHTA INDICA* IN ALBINO RATS

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Abstract

The aim of this study was the screening of anxiolytic & antidepressant potential of hydro-alcoholic leaves extract of *Azadirachta indica* (Neem) in albino rats. The fresh leaves of Neem were collected from Amethi region; identified and authenticated by botanist at MJP Rohilkhand University, Bareilly. The hydro-alcoholic extract was obtained by maceration in 1:1. The rats were administered *p. o.* the hydro-alcoholic leaves extract of *A. indica* in 100mg/kg and 200mg/kg, once daily for 15 days. The Elevated Zero Maze, Light/Dark Arena Test, Forced swimming test and Locomoter activity (Actophotometer) models were used for the screening of anxiolytic and antidepressant potential. Neem (*A. indica*) leaves at the both doses (100 & 200 mg/kg) exhibited statistically significant anxiolytic & antidepressant effect in all the parameters observed when compared with control and standard (fluoxetine hydrochloride) group. The result clarified that after once daily treatment up to 15 days the hydro-alcoholic leaves extract of *A. indica* at lower dose (100 mg/kg) exhibits most significant anxiolytic & anti-depressant activity than higher dose (200 mg/kg). This research concludes that small dose has more significant potential and safety than higher dose. This research further suggests isolation and purification of active moiety and determination of mechanism of action by which these pharmacological potentials are exhibited.

Keywords: *Azadirachta indica, antidepressant, anxiolytic, fluoxetine*

Introduction

Depression and anxiety both are the most common affective mental illnesses that require an adequate medical treatment. A 25% of human populations are affected by psychological disorders at any point during their lives [1]. In the beginning of 1980s, drugs related interactions and substances abuse increased the psychiatric disorders in inmates, so this time was known as 'war on drugs' [2]. According to World Health Organization (W.H.O.) study, approximately 1 in 4 American adult was suffered with mental disorders in America than in other countries world-wide [3]. It was demonstrated that at any moment about 3-5% population is depressed and 10% of people are thought to be depress during their lives globally [4]. According to W.H.O., 121 millions of world population suffered from clinical depression disorder [5]. According to W.H.O. almost 3000 population undergoes suicidal death and such patients attempt suicide 20 or more than it to finish his/her life. Depression is a pervasive mental disorder world-wide [6]. Depression arises due to functional deficiency in levels of serotonin, dopamine and MAOs neurotransmitters in the brain. Therefore, such drugs that elevate the levels of these neurotransmitters in CNS exhibit antidepressant potential [7]. The clinical diagnosis of depression is relevant to depressed mood and pleasure [8]. Anxiety disorder is among the most prevalent mental illnesses in the general population. The prevalence rate of anxiety illnesses is estimated 18.5 per 1000 population throughout the India [9]. *Azadirachta indica A. Juss (A. indica)* commonly known as neem (family- Meliaceae) has been the most useful traditional plant. Being the most investigated tree globally, believed to be highly hopeful tree of 21st century [10]. About 5000 neem trees are grown over 10 km plains of Arafat to make shade for Muslims during Hajj [11]. Two species of *Azadirachta* have been investigated yet. *Azadirachta indica* is homegrown to Indian subcontinent and *Azadirachta excelsa* Kack that confirmed to Indonesia and Philippines [12]. Recently, studies done over anxiolytic activity of neem leaves extract has shown the acute anxiolytic effect through ultra-short term treatment in albino rats when compared with Diazepam and Buspirone. However anxiolytic and anti-depressant activities through long term treatment of Neem leaves extract have not been studied yet. Therefore, the present study was designed to determine the anxiolytic and anti-depressant potential through long term treatment of hydro-alcoholic leaves extract of Neem *(Azadirachta indica)* and to compare with control and standard fluoxetine hydrochloride (SSRIs) group.

Methods

Animals

The Wistar Albino rats (either sex) were obtained from the departmental animal house of M. J. P. Rohilkhand University Campus Bareilly.

Experimental Requirements (See table 1)

Collection, Identification and authentication of the plant

Fresh leaves of neem (*A. indica)* were collected from Amethi region, Uttar Pradesh. The leaves of *A. indica* were identified & authenticated by the botanist at MJP Rohilkhand University under the specimen no. RU/PS/2016/528.

Extraction of plant

The leaves were washed with purified water to remove dirt and air dried in shadow for a month then crushed in coarse powder. It was weighed (300 g) and cold macerated (1.5 Lt.) in hydro-alcoholic (1:1) solution for 15 days with gradual stirring. Macerate was filtered using cotton plug then finally with whatman filter paper. The filtrate was evaporated under water-bath at 40°C till leaving solid residue. Thus finally we obtained a highly hygroscopic greenish leaves extract powder with 6.78 percentage yield.

$$\text{Percentage yield} = \text{Practical yield}/\text{Theoretical yield} \times 100$$

$$\text{Percentage yield} = 20.36/300 \times 100 \cong 6.78$$

Preparation of animals

Wistar albino rats (either sex) were obtained from the departmental animal house of M.J.P. Rohilkhand University Campus, Bareilly. Animals were kept under controlled environment 12 h dark/light cycles (temp. 23±2°C) and have free access to food and purified water *ad libitum*. Animals handling was

proceed according to the Institutional Animals Ethics Committee.

Acute Oral Toxicity

The doses of Neem (*A. indica*) leaves extract were selected according to limit-test of Organization for Economic Co-operation and Development (OECD) Guideline No. 423. Animals were kept under controlled environment 12 h dark/light cycles (temp. 22±3°C) and free access to food and purified water *ad libitum* for 5 days. Three rats (overnight fasting) were used for each fixed dose level 5, 50, 300 & 2000 mg/kg. On 300 mg/kg body weight treatment each 3 rats were found dead during 4- 24 h. Therefore from the above observation dose selected as 100 and 200 mg/kg body weight [13].

Experimental design

Wistar Albino rats (either sex) were weighed and divided into 4 groups, each consisting of 6 animals. They were treated once per day for 15 days as follows-

Group I (Control) : Administered distilled water (15 ml/kg) body weight *p. o.*

Group II (Standard) : Administered fluoxetine hydrochloride (15 mg/kg *p. o.*) suspended in distilled water.

Group III (Test a) : Administered hydro-alcoholic leaves extract of *A. indica* (100 mg/kg *p. o.*) suspended in distilled water.

Group IV (Test b) : Administered hydro-alcoholic leaves extract of *A. indica* (200 mg/kg *p. o.*) suspended in distilled water.

Screening Protocol

The pharmacological screening of test drug was carried out using following models.

1. Elevated Zero Maze Test

The elevated zero maze consists a 5 cm wide circular pathway that is elevated 27 cm from the floor. The diameter of maze kept 65 cm. The circular pathway was divided into 4 quadrants; 2 open quadrants and 2 closed quadrants which had wall (27 cm high). Rats were placed facing towards one of the closed quadrants at each trial. Rats were allowed to explore the apparatus for 5 minutes. No. of entries and time spent in open quadrants were recorded till 5 minutes [14].

2. Light-Dark Arena Test

In light-dark arena model, a 100 Watt bulb was placed 30 cm above the floor of box. Rats were kept in centre of light arena and exposed for 5 minutes. No. of entries and time spent in light arena were recorded till 5 minutes. It was cleansed every time before keeping a new animal [14].

3. Forced Swimming Test

Rats were dropped in glass (30×20 cm) filled water at depth of 15 cm and temperature maintained at 30°C. Rats were allowed to force swim for 5 min. The total mobility time was recorded in sec during 5 min using stopwatch [15].

4. Locomotor Activity

Turned on the Actophotometer to check and make sure that all the photo cells are working properly for accurate recording. Rats were placed individually in the activity cage for 10 min. Activity score was recorded for each animal till 10 min. Finally, motor activity was observed and compared with control/standard [14].

Results

Elevated Zero Maze Test

In elevated zero maze test model, table 5.1 depicts the anxiolytic effect of the administered drugs. It exhibited the statistically significant anxiolytic activity when compared with control and standard group. However lower dose (100 mg/kg) demonstrated the increased *mean no. of entries* & *% of mean time spent* in open arms than higher dose 200 mg/kg treated group (Table 2).

Light-Dark arena test

In Light-Dark arena model, table 5.2 depicts the anxiolytic effect of the administered drug. It exhibited the statistically significant anxiolytic activity when compared with control and standard group. However lower dose (100 mg/kg) demonstrated the increased *mean no. of entries* & *% of mean time spent* in light arena than higher dose 200 mg/kg treated group (Table 3).

Forced Swimming Test

In forced swimming test model, table 5.3 depicts the antidepressant effect of administered drugs. It

exhibited the statistically significant antidepressant potential when compared with control and standard group. However, lower dose (100 mg/kg) demonstrated the increased *mean mobility time* than the higher dose (200 mg/kg) treated group (Table 4).

Locomotor Activity

In actophotometer test model, Table 5 depicts the antidepressant potential of test drug. It exhibited statistically significant antidepressant potential when compared with control and standard group. However, lower dose (100mg/kg) demonstrated the decreased locomotor activity than the higher dose (200mg/kg) treated group (Table 5).

Discussion

In Elevated Zero Maze model- the mean No. of entries and time spent in open arms were found 9.16± 0.47 vs. 154.33± 0.99 in Standard group. Other treated groups were increased the mean no. of entries & mean time spent in open arms comparatively the control group. Test group treated with 100 mg/kg demonstrated statistically significant increase in mean no. of entries & mean time spent in open arms 8.33± 0.49 vs. 121.5± 0.88 than Test group treated with 200 mg/kg 7.5± 0.42 vs. 117± 0.85 when compared with Control group 4.16± 0.30 vs. 66.83± 0.70. The individual albino rats were explored to Elevated Zero Maze apparatus for 300 sec. In Light-Dark arena model- the mean no. of entries and time spent in light arena were found 9± 0.57 vs. 156.83± 0.83 in Standard group. Other treated groups were increased the mean no. of entries & mean time spent in light arena comparatively the control group. Test group treated with 100 mg/kg demonstrated statistically significant increase in mean no. of entries & mean time spent in light arena 7.83± 0.79 vs. 130.66± 0.76 than Test group treated with 200 mg/kg 6.83± 0.60 vs. 124.5± 0.67 when compared with Control group 5.16± 0.47 vs. 70.33± 0.71. The individual albino rats were explored to Light-Dark arena for 300 sec. In Force Swimming model, all the treated groups were significantly increased the mean of mobility time except control group 250.66± 0.76. Test group treated with 100 mg/kg exhibited statistically significant increase in mean of mobility time 280.5± 0.88 than test group treated with 200 mg/kg 275.83± 0.94 when compared with Standard group 289.5± 0.99. Increase in mean of mobility time indicates the anti-depressant potential of the test drug at both dose levels. The individual albino rats were explored to force swimming test apparatus for 300 sec. In Locomotor Activity model, the mean of locomotor activity (scores) was found 157± 0.85 in Control group. All the other treated groups were significantly decreased the motor activity in albino rats. Test group treated with 100 mg/kg demonstrated statistically significant decrease in mean of locomotor activity (scores) 95.5± 0.92 than test group treated with 200 mg/kg 102.33± 0.80 when compared with Standard group 95.5± 0.92. The individual albino rats were explored to Digital Actophotometer for 10 min. All these effects (summarized in table 2, 3, 4) are believed due to presence of flavonoids.

Conclusion

On the basis of above results, it concluded that hydro-alcoholic leaves extract of *Azadirachta indica* (Neem) has significant anxiolytic and antidepressant potential. Both the dose levels 100 & 200 mg/kg of *A. indica* demonstrated statistically significant anxiolytic and antidepressant effect in all the parameters observed when compared with control. Although the mechanism behind its anxiolytic and antidepressant potential is unknown but the observed activity can be assigned due to plant components having similar structure to SSRIs. So it is necessary to undergo further research to isolate, purify and identify the active chemical constituent in leaves of neem, responsible for anxiolytic and antidepressant potential and also determine the mechanism of action.

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Table 1: **Experimental requirements**

S.N.	***Chemicals***	***Instruments/ Apparatus***
1.	Hydro-alcoholic leaves extract powder of neem	Water bath, Desiccator & Actophotometer
2.	Fluoxetine hydrochloride (Prozac) Mfd. By Eli Lilly & Co.	Elevated Zero Maze
3.	Ethanol	Forced Swimming Test apparatus
4.	Distilled water	Light/Dark Arena

Table 2: **A comparative observation of *mean no. of entries* & *% of mean time spent* in open arms of different treated groups**

S. N.	Treatment	Mean no. of entries in open arms	Mean time spent in open arms (sec)	% of Mean time spent in open arms (sec)
1.	*Control*	4.16±0.30**	66.83±0.70	22.27± 0.23**
2.	*Standard*	9.16± 0.47***	154.33± 0.99	51.44± 0.33***
3.	*Test a*	8.33± 0.49***	121.50± 0.88	40.50± 0.29***
4.	*Test b*	7.50± 0.42***	117.00± 0.85	39.00± 0.28***

Level of significance: *
(n= 6, values were expressed in Mean± SEM and statistically significant at P<0.05 compared to control.

Table 3: **A comparative observation of *mean no. of entries* & *% of mean time spent* in light arena of different treated groups**

S. N.	Treatment	Mean No. of entries in light arena	Mean time spent in light arena (sec)	% of Mean time spent in light arena (sec)
1.	*Control*	5.16± 0.47**	70.33± 0.71	23.44±0.23**
2.	*Standard*	9.00± 0.57***	156.83± 0.83	52.27±0.27***
3.	*Test a*	7.83± 0.79*	130.66± 0.76	43.55±0.25***
4.	*Test b*	7.50± 0.60*	124.50± 0.67	41.50±0.22***

Level of significance: *
(n= 6, values were expressed in Mean± SEM and statistically significant at P<0.05 compared to control.

Table 4: **A comparative observation on *mean mobility time* of different treated groups**

S. N.	Treatment	Mean mobility time in 5 min (sec)
1.	*Control*	250.66± 0.76***
2.	*Standard*	289.5± 0.99***
3.	*Test a*	280.5± 0.88***
4	*Test b*	275.83± 0.94***

Level of significance: *
(n= 6, values were expressed in Mean± SEM and statistically significant at P<0.05 compared to control.

***Table 5*: A comparative observation on *mean locomotor activity (scores)* of different treated groups**

S. N.	Treatment	Locomotor activity (scores) in 10 min (sec)
1.	*Control*	157± 0.85***
2.	*Standard*	95.5± 0.92***
3.	*Test a*	102.33± 0.80***
4.	*Test b*	110± 0.85***

Level of significance: *
(n= 6, values were expressed in Mean± SEM and statistically significant at P<0.05 compared to control.