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EVALUATION OF NEUROLEPTIC POTENTIAL OF HYDROALCOHOLIC LEAVES EXTRACT (HLE) OF *AZADIRACHTA INDICA* (NEEM) IN ALBINO RATS

***[1]Najeebullah, [2]N. Trilochana and [3]Ashutosh Yadav**

[1]Research Scholar, Institute of Pharmaceutical Sciences and Research, Unnao (UP) IN.
[2]Associate Professor, Institute of Pharmaceutical Sciences and Research, Unnao (UP) IN.
[3]Faculty, Lucknow Model College of Pharmacy, Lucknow.

***Corresponding Author: Najeebullah**
Research Scholar, Institute of Pharmaceutical Sciences and Research, Unnao (UP) IN.

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ABSTRACT

Psychosis is a term used to describe disorders that affect the mind and create a loss of contact with reality. Women are 1.5-2 times more likely than men to suffer from anxiety disorders. Present study was based on evaluation of neuroprotective effect of hydroalcoholic leaves extract (HLE) of *A. indica* in Wistar albino rats by using diverse types of animal models to confirm its actual pharmacological potential. Leaves of *M. indica* was obtained from the Unnao reason. It was identified and authenticated by a botanist and extracted by percolation process. Rats were divided into group 1 (normal saline), group 2 (Apomorphine 5 mg/kg), group 3 (Pregabalin (10mg/kg), group 4a & 4b (Aq. AI leaves extract- 200mg/kg & 400mg/kg), group 5a & 5b (Eth. AI leaves extract- 200mg/kg & 400mg/kg) and group 6a & 6b (Meth. AI leaves extract- 200mg/kg & 400mg/kg). Different parameters were evaluated i.e., Apomorphine-induced stereotypy, Haloperidol induced-catalepsy, Climbing behavior and Neurotransmitter measurements. In results, it significantly decreased the climbing behavior, stereotyped behavior and elevated levels of neurotransmitters in rats. It concludes that AI has potential neuroprotective behaviour at the all the doses used but highest was observed in ethanolic extract- might be due to better solubility and constituents release from the A. indica leaves in percolation. Future research suggests, to identify and isolate the responsible element for the pharmacological potential and make the suitable dosage form the make available in the market and society in the cure of this deadly affective mental disorder.

KEYBOARDS: Antipsychotic, hepatoprotective, neem, *Azadirachta indica*, Catalepsy.

INTRODUCTION

Psychosis is a term used to describe disorders that affect the mind and create a loss of contact with reality. Delusions and hallucinations, or seeing/ hearing something that does not exist, are common symptoms of psychosis. Incoherent or nonsensical speech, as well as improper behavior for the situation, are other indicators (Schizophrenia, Mental Health Information, 2022). Women are more likely than men to acquire emotional disorders or anxiety during adolescence. Women are 1.5-2 times more likely than men to suffer from anxiety disorders (Thibaut, 2017).

Psychotic symptoms, like delusions and hallucinations, can appear gradually and develop over time, or they might appear suddenly. People suffering from psychosis may be unaware that their sensations are abnormal. What is going on in their heads is extremely real to them. A primary psychiatric disorder, substance abuse, or another neurologic or medical issue can all cause psychosis. Decreased prefrontal, superior, and medial temporal grey matter, as well as other brain abnormalities, have been linked to first-episode psychotic illnesses (Pantelis et al. 2003). Despite more than a century of investigation, scientists are still unable to pinpoint the exact cause of schizophrenia (Siever & Davis, 2004).

The neem tree is a member of the Meliaceae family, which is abundant in tropical & semitropical regions such as India, Pakistan, Bangladesh, and Nepal. It is a rapidly tree that can reach 20–23 m in height and has a straight trunk with a diameter of 4-5 ft. The leaves are complex, imparipinnate, and have 5–15 leaflets each. Its fruits are greenish drupes that turn golden yellow when ripe between June and August (Alzohairy, 2016).

In recent years, Neem-derived extracts have been demonstrated to be effective in a variety of applications, including insect repellents, anti-inflammatory supplements, diabetic control, and even cancer treatment (Islas et al. 2020).

Azadirachtin is the most important active ingredient, followed by nimbin, nimbidin, nimbolinin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol are all found in the leaves (Ali, 1993; Hossain et al. 2011; Kokate, 2010).

On the basis of above literature survey, I found that previous researches demonstrate the antipsychotic effect of leaves extracts of *A. indica* using animal models but in a limited manner and not explicated well.

So, this research focuses on evaluation of neuroprotective effect of hydroalcoholic leaves extract (HLE) of *A. indica* in Wistar albino rats by using diverse types of animal models to confirm its actual pharmacological potential.

MATERIALS AND METHODS

Materials

Hydroalcoholic leaves extract (HLE) of *Azadirachta indica*, Pregabalin (API), Wistar rats (either sex), fridge-dryer or rotatory evaporator/ Water bath, ethanol, distilled water, beaker, conical flask.

Collection, Identification & Authentication of plant

Leaves of *M. indica* was obtained from the Unnao reason. It was identified and authenticated by a botanist. The leaves are washed making dust-free and dried at room temperature or shade. The dried leaves are rendered into coarse powders and then finally into fine ones. The powder is weighed and extracted separately into 3 solvents i.e., distilled water, ethanol and methanol using percolator. A rotating evaporator is used to dry the brownish, semisolid extract obtained under partial vacuum (Khan et al. 2020).

$$\text{percent yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100\%$$

Fig. 2.1 Dried leaves of *A. indica*.

Fig. 2.2: Powdering of *A. indica* leaves.

Fig. 2.3: Weighing of AI leaves (dried).

Extraction of AI Leaves

The powder is weighed and soaked separately into 3 solvents i.e., distilled water, ethanol and methanol for fifteen days with gradual stirrings.

Cold percolation

A percolator is a tall cylindrical jar with a cone bottom and a constructed fake bottom with a filter cloth that is used to remove plant material. For solvent removal from the marc, the percolator is attached to a condenser and a receiver. It was extracted using water, ethanol and methanol solvent separately.

Water/Ethanol/Methanol is used to macerate the powdered material, which is then poured into a tall column. The powdered substance is immersed in cold water until it is completely submerged. Water-soluble

components are allowed to reach equilibrium in the water after 24 hours. The aqueous enhanced extract is condensed to a specific concentration in multiple-effect evaporators. This concentrated extract is diluted and then ready to be used as a medicine (Handa et al. 2008).

Preparation of animals

Animal House, Institute of Pharmaceutical Sciences and Research (IPSR), Unnao provided albino rats of either sex weighing 150–200 g. The animals are kept in good health, with room temperatures of 25°C and a 12-hour light/dark cycle. The relative humidity is kept at 44–56 percent, and the rats are provided a regular rodent diet and free access to water. The animals were kept on fasting but have free access to water until 1 hour before the ulcers are induced (Bhajoni et al. 2016).

Group design

All the rats are divided into 4 groups (n=6) as followings-

Group 1: Rats are administered only normal saline once a day for 15 days.

Group 2: Rats are administered Apomorphine (APO) (5mg/kg) once a day for 15 days.

Group 3: Rats are administered Pregabalin (10mg/kg) orally, for 15 days.

Group 4a &4b: Rats are administered aqueous leaves extract of *A. indica* 200mg/kg & 400mg/kg respectively p. o. for 15 days.

Group 5a & 5b: Rats are administered ethanolic leaves extract of *A. indica* 200mg/kg and 400mg/kg per orally up to 15 days.

Group 6a & 6b: Rats are administered methanolic leaves extract of *A. indica* 200mg/kg and 400mg/kg per orally up to 15 days.

Protocols

1. Apomorphine-induced stereotypy

The animals were separated into four groups (n = 6), with Group I serving as the control group, receiving normal saline (per oral). Pregabalin 10 mg/kg, i. p. was given to Group II as a drug control. HLE of AI was given to Group III as 200mg/kg and group IV as 400mg/kg at the 60 min before administration apomorphine (5mg/kg, s. c.). stereotyped behavior is a result of stereotyping. The rats were housed in individual cages and watched for 10 seconds. The following scoring method was used to determine the severity of stereotyped activity: 0, asleep or still; 1, energetic; 2, predominantly active but with intervals of stereotyped sniffing & rearing; 3, fixed stereotyped behavior such as sniffing, rearing, or head bobbing, but with locomotor activity still present; 4, constant stereotyped activity maintained at one location; 5, constant stereotyped activity maintained at one location; 6, constant stereotyped activity maintained at one location; 7, constant stereotyped activity maintained at one location; 8, constant stereotyped activity maintained at one location; 9, constant stereotyped activity maintained at one location; 5, stereotyped activity with bursts of licking, gnawing, and biting; 6, persistent licking of cage grids; 7, consistent biting of cage grids.

After apomorphine treatment, which are intervals that allows you to track the behavioral effects of apomorphine over time, the presence or absence of movement, rearing, sniffing, licking, and chewing will observe at every 10, 20, 30, 45, 60 & 90 min (Amos et al. 2003).

2. Haloperidol induced-catalepsy

The animals were separated into three groups (n = 6), with Group I serving as the control group, receiving normal saline p. o. The AI was given to Group III and IV animals at doses of 200 and 400 mg/kg, (orally) 60 minutes before the HAL (1 mg/kg, i. p.) was given. The following is the severity of the catatonic response that was observed: Stage I, rats move normally when placed on the table, score = 0; stage II, rats move only when touched or pushed, score = 0.5; stage III, a rat placed on the table with front paws occasionally on a 3 cm high frame fails to correct the posture in 10 seconds, score = 0.5 for each paw for a total of 1 for this stage; stage IV, a rat placed on the table with front paws alternately on a 9 cm block fails to remove. Thus, the maximum potential score for a single rat would be 3.5, indicating total catatonia. The total catatonia score will be calculated at every 10, 20, 30, 45, 60 & 90 min (Praveen & Brij, 2005; Kulkarni, 1999).

3. Climbing behavior

Each mouse was placed individually in a vertical wire-mesh stick cage (diameter 12 cm; height of 14 cm) for the determination of climbing behavior every 10 minutes for a period of 30 minutes, and evaluated as follows: 0 = four paws on the floor, 1 – forefeet holding the bars, 2 – four feet holding the bars. Rats were individually habituated in the vertical wire-mesh stick cage for 30 minutes before apomorphine induction to reduce scoring error owing to exploratory behavior (Vogel, 2008; Durg et al. 2015).

4. Neurotransmitter measurements

Following behavioral evaluations, all animals were slaughtered and their brains were dissected for neurotransmitter estimations (dopamine, noradrenaline, and serotonin; expressed in pg/mg of wet brain tissue) according to normal protocol (Chitra et al. 2014; Maheshwari, 2015).

RESULTS AND DISCUSSION

1. *Apomorphine-induced stereotypy*

When stereotype score was seen in control group it was observed as 2.31±0.41** and 3.12±0.12** at 10 min and 90 min respectively. It clearly denotes, in contrast to APO control group that showed the same as 2.67±0.37** and 3.53±0.30** at 10 and 90 min respectively which is far different from the *A. indica* treated animals.

In all the groups of animals, A. indica showed excellent neuroprotective behavior when observed in comparison with control and apomorphine control groups. At 45 min, pregabalin group showed stereotype score as 0.82±0.49** and Aq. AI as 1.94±0.33** (200mg/kg) and 1.10±0.71*** (400mg/kg). It we see, the effects of Eth. AI then it was found as 1.61±0.57** and 1.05±0.41** at 100mg/kg and 200mg/kg respectively which is much closer to standard group. So, it may be concluded that ethanolic extract has much significant potential than two other extracts used i.e., aqueous and methanolic.

Table 1: Stereotype Score of APO, Pregabalin and AI.

Treatment	Stereotype Score (Mean ± SEM)					
	10 min	20 min	30 min	45 min	60 min	90 min
Normal Saline	2.31±0.41**	3.81±0.18***	4.37±0.52**	5.24±0.15***	4.43±0.38**	3.12±0.12**
APO (5mg/kg)	2.67±0.37**	3.94±0.49**	5.23±0.12**	5.82±0.61**	5.10±0.13***	3.53±0.30**
***Pregabalin* (10mg/kg)**	0.47±0.23**	0.94±0.19**	1.20±0.57**	0.82±0.49**	0.52±0.31***	1.11±0.31**
***Aq. AI* (200mg/kg)**	2.12±0.51**	2.70±0.32***	2.14±0.41**	1.94±0.33**	1.64±0.60**	2.43±0.23***
***Aq. AI* (400mg/kg)**	1.76±0.24**	1.69±0.38***	1.32±0.15***	1.10±0.71***	1.47±0.52**	1.97±0.57**
***Eth. AI* (200mg/kg)**	2.54±0.37***	2.81±0.27**	2.35±0.19**	1.61±0.57**	1.94±0.24***	2.37±0.81**
***Eth. AI* (400mg/kg)**	2.31±0.26**	1.67±0.11**	1.54±0.24***	1.05±0.41**	1.27±0.33***	2.16±0.71**
***Meth. AI* (200mg/kg)**	2.62±0.61***	2.17±0.21**	2.38±0.39**	1.70±0.71**	2.21±0.19***	2.72±0.47**
***Meth. AI* (400mg/kg)**	2.41±0.31***	1.80±0.40***	1.32±0.14***	1.14±0.62**	1.60±0.12**	2.41±0.31***

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

2. Haloperidol-induced catalepsy

The maximum inhibitory catalepsy score was seen in group 3 treated with Haloperidol, as 11.21±1.37** which so far from the apomorphine treated animals. Ethanolic extract of AI showed catalepsy score as 31.27±1.62** and 31.27±1.62** at the dose of 200mg/kg and 400mg/kg respectively that are much closer to the standard drug treated group when compared with all other group of animals.

Whereas, highest catalepsy score was recorded at 60 min in apomorphine treated group as 78.23±1.29***. Catalepsy inhibitory role was observed in all the AI extracts but it was highest in ethanolic and lowest in methanolic.

Table 2: Catalepsy Score of APO, Haloperidol and AI.

Treatment	Catalepsy Score (Mean ± SEM)					
	10 min	20 min	30 min	45 min	60 min	90 min
Normal Saline	68±1.31**	73.41±1.17**	71.50±1.23**	69.17±1.16**	74.13±1.31**	70.20±1.26**
APO (5mg/kg)	72±1.51**	75.61±1.16**	74.30±1.55**	76.27±1.14**	78.23±1.29***	65.40±1.54***
***Haloperidol* (1mg/kg)**	51.41±1.27**	53.14±1.18**	55.51±1.52**	53.25±1.31**	53.29±1.31**	11.21±1.37**
***Aq. AI* (200mg/kg+ HAL**	54.37±1.51**	54.52±1.35**	56.24±1.61**	58.20±1.26**	61.34±1.41**	53.51±1.27**
***Aq. AI* (400mg/kg) + HAL**	61.26±1.24**	60.29±1.11**	58.12±1.14***	58.16±1.36**	57.37±1.50**	51.43±1.36**
***Eth. AI* (200mg/kg) + HAL**	53±1.33***	55.22±1.25**	57.12±1.21**	59.21±1.57**	56.35±1.31**	31.27±1.62**
***Eth. AI* (400mg/kg) + HAL**	60±1.23**	61.31±0.93**	59.43±1.24***	57.25±1.42**	56.47±1.39**	56.34±1.61**
***Meth. AI* (200mg/kg) + HAL**	55±1.61***	54.34±1.29**	59.14±1.19**	57.50±1.60**	56.21±1.17**	54.47±1.38**
***Meth. AI* (400mg/kg) + HAL**	59.41±0.93**	62.30±1.51**	59.26±1.18**	58.71±1.64**	58.92±1.53**	57.21±1.37**

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

3. Apomorphine- induced climbing behaviour

Climbing time was noted as 16.17±0.32* and 22.31±0.61** in normal saline and apomorphine treated rats. Similarly, cumulative climbing index was observed 17.21±0.22** (group 1) and 21.42±0.39** (APO group). Climbing time was significantly found decreased as 6.19±0.17** and cumulative climbing index as 21.42±0.39** which was lowest among all the treatments. After standard drug, maximum inhibitory role was seen in ethanolic extract of AI as 8.28±0.24** when compared with other extracts such as methanolic or aqueous.

Table 3: Climbing time of APO, Pregabalin and AI.

Treatment	Climbing time (30 min)	Cumulative climbing index
Normal Saline	16.17±0.32*	17.21±0.22**
APO (5mg/kg)	22.31±0.61**	21.42±0.39**
***Pregabalin* (10mg/kg)**	6.19±0.17**	7.35±0.71**
***Aq. AI* (200mg/kg)**	15.52±0.29*	14.24±0.43**
***Aq. AI* (400mg/kg)**	9.24±0.38***	8.56±0.46**
***Eth. AI* (200mg/kg)**	14.20±0.11***	13.41±0.12***
***Eth. AI* (400mg/kg)**	8.28±0.24**	8.17±0.50**
***Meth. AI* (200mg/kg)**	14.34±0.14**	12.28±0.17**
***Meth. AI* (400mg/kg)**	8.48±0.10**	7.51±0.13**

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

4. Climbing behaviour score

Similarly, on the basis of climbing time reduction, climbing score was noted out of 5. In 30 min, climbing score was found as 1.16±0.24* in the ethanolic extract of AI which was lowest when compared with control as 3.54±0.32** and APO control group as 3.95±0.11** in 30 min.

Table 4: Climbing behaviour Score.

Treatment	Climbing Score out of 5 (Mean ± SEM)		
	10 min	20 min	30 min
Normal Saline	2.94±0.37**	4.41±0.21**	3.54±0.32**
APO (5mg/kg)	3.23±0.53**	4.47±0.37**	3.95±0.11**
***Pregabalin* (10mg/kg)**	1.23±0.32***	1.12±0.15*	0.24±0.27***
***Aq. AI* (200mg/kg)**	2.60±0.51**	3.25±0.62***	1.46±0.13***
***Aq. AI* (400mg/kg)**	2.01±0.26**	1.36±0.80**	0.57±0.17**
***Eth. AI* (200mg/kg)**	2.42±0.23**	2.27±0.42*	1.16±0.24*
***Eth. AI* (400mg/kg)**	1.83±0.31**	1.03±0.47**	0.49±0.67***
***Meth. AI* (200mg/kg)**	2.34±0.29**	3.07±0.38**	1.35±0.35**
***Meth. AI* (400mg/kg)**	2.15±0.11***	1.47±0.12***	0.37±0.66***

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

5. Neurotransmitter Measurements

In neurotransmitters measurements model, total 6 groups of rats were used to screen-out neuroprotective potential in contrast to standard and control group. Group 1 was kept as control that was served normal saline only. Group 2 was fed with apomorphine (5mg/kg) and served as APO control group. Group 3 was treated with standard neuroprotective drug- Pregabalin (10mg/kg) whereas, group 4a & 4b was administered Aqueous extract of *A. indica* (Aq. AI) as 200mg/kg & 400mg/kg respectively. Group 5a and 5b was given Ethanolic leaves extract of *A. indica* in the dose of 200mg/kg and 400mg/kg respectively. In last, group 6a and 6b was served methanolic leaves extract of *A. indica* in the dose of 200mg/kg and 400mg/kg respectively. Thus, all total 6 groups were used, each containing 6 rats.

Table 5: Level of neurotransmitters.

Treatment	Wet brain tissue concentration (pg/mg)		
	Dopamine	Noradrenaline	Serotonin
Normal Saline	2300±0.02**	540±0.52**	740±0.41**
APO Control	2900±0.32**	600±0.39***	800±0.49***
***Pregabalin* (10mg/kg)**	1500±0.12***	350±0.71**	410±0.60***
***Aq. AI* (200mg/kg)**	2200±0.54**	450±0.13**	470±0.37**
***Aq. AI* (400mg/kg)**	1800±0.11**	400±0.17**	450±0.27***
***Eth. AI* (200mg/kg)**	2000±0.29**	430±0.33***	500±0.16***
***Eth. AI* (400mg/kg)**	1700±0.51***	370±0.10***	430±0.25**
***Meth. AI* (200mg/kg)**	2300±0.62**	440±0.42***	490±0.10***
***Meth. AI* (400mg/kg)**	1800±0.15**	390±0.55**	450±0.19**

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6).

Inhibitory action in the levels of neurotransmitters was found maximum in pregabalin treated animals as 1500±0.12***, when compared with APO control group as 2900±0.32** in the case of dopamine. Similarly, a prominent reduction in levels of noradrenalin and serotonin was estimated.

As the results indicate in all the models, AI has prominent neuroprotective effect at lower and higher doses (100mg/kg & 200mg/kg). But ethanolic extract showed much significant neuroprotective behavior it might be due to better release of constituents from the leaves of AI. Whereas, action produced by methanolic extract and aqueous extract was also significant but not as much as ethanolic extracts.

CONCLUSION

AI inhibited apomorphine-induced climbing and stereotyped behaviour in mice, and these effects may be partially mitigated by dopaminergic and serotonergic pathway inhibition, as well as noradrenergic neuron inhibiting activity. To understand the precise manner of its antipsychotic activity, more research is needed.

It concludes that AI has potential neuroprotective behaviour at the all the doses used but highest was observed in ethanolic extract- might be due to better solubility and constituents release from the A. indica leaves in percolation.

Future research suggests, to identify and isolate the responsible element for the pharmacological potential and make the suitable dosage form the make available in the market and society in the cure of this deadly affective mental disorder.

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Nil.

CONFLICT OF INTEREST

None.

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