IJPSR (2013), Vol. 4, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 March, 2013; received in revised form, 21 April, 2013; accepted, 24 June, 2013

EVALUATION OF ANTIPSYCHOTIC ACTIVITY OF ETHANOLIC EXTRACT OF *NARDOSTACHYS JATAMANSI* ON WISTAR ALBINO RAT

Rajiv Jash*[1], K. Apanna Choudary [2] and D.S.N.B.K. Prasanth [3]

Department of Pharmaceutical Sciences, Singhania University [1], Pacheri Bari, Rajasthan
St. Ann's College of Pharmacy [2], Cantonment road, Vizianagaram, Andhra Pradesh, India
Department of Pharmacology, Sri Vishnu College of Pharmacy [3], Bhimavaram, Andhra Pradesh, India

Keywords:

Neuroleptic, Dopamine, Striatum, Cortex, Psychosis, Dyskinesia

Abbreviations:

NJ- *Nardostachys jatamansi*

Correspondence to Author:

Rajiv Jash

C/o M. Rama Rao, MIG-1-70, Housing Board Colony, -834201 Andhra Pradesh

E-mail: jashrajiv@gmail.com

ABSTRACT: An increased inclination has been observed for the use of herbal drugs in chronic and incurable diseases. Treatment of psychiatric diseases like Schizophrenia is largely palliative and more importantly a prominent adverse effect prevails with the majority of antipsychotic drugs, which are the extrapyramidal motor disorders. This study was a trial to evaluate the neuroleptic activity of the ethanolic extracts of Nardostachys Jatamansi with different antipsychotic animal models. Two doses of the extract (100 and 200mg/kg) were used for this study with 5 different animal models. After that, the concentration of the dopamine neurotransmitter was estimated in two different regions of the brain viz. Frontal cortex and Striatum. The result of the study indicated a significant reduction of amphetamine induced stereotype and conditioned avoidance response for the extracts compared with the control group, but did not have any significant effect in phencyclidine induced locomotor activity and social interaction activity. However the extract showed minor signs of catalepsy compared to the control group. The study also revealed that the neuroleptic effect was due to the reduction of the dopamine concentration in the frontal cortex region of the rat brain. The results largely pointed out the fact that the extract may be having the property to alleviate the positive symptoms of Schizophrenia by reducing the dopamine levels of dopaminergic neurons of the brain. The estimation of dopamine in the two major regions of brain indicated the alteration of dopamine levels was the reason for the antipsychotic activity as demonstrated by the different animal models.

INTRODUCTION: Incidence of schizophrenia is high which accounts for almost 1 in every 276 people [1] or precisely 2.5 million people, throughout the world today.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(7).2730-36

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(7).2730-36

Schizophrenia and psychosis has captured the headlines increasingly for the past few years. Anti-psychotic drugs which are in use today, the safety profile is not so promising considering the fact that it has to be continued for a few years. The significant and serious adverse effect of these drugs is the extrapyramidal side effects which includes akathesia, acute muscle dystonia, and tardive dyskinesia [2]. Over the long term, these antipsychotics may cause dopaminergic pathways in the brain permanently dysfunctional [3].

They may lead to severe movement disorders like tardive dyskinesia, tardive psychosis, and global cognitive decline tardive dementia [2, 3]. There has been enough evidence as suggested by the recent MRI scans of Schizophrenia patients that antipsychotics even are responsible for the shrinkage of the basal ganglia region of the brain [3]. Keeping all the above facts in mind, this study was a trial for exploration of the herbal formulations which can be used for the treatment of schizophrenia patients.

Demand for Herbal drugs is ever increasing. Herbal drugs are known to have very minimal adverse effect and its well worth a therapy for chronic CNS diseases like Psychosis which is virtually incurable. India is a country where ayurvada has been practiced from the Vedic ages successfully. Such a herbal plant is N. Jatamansi which belongs to the family Valerianceae found widely in all parts of India. The roots and the rhizome extracts (ethanolic) and its fraction have been studied for improvement of cognition, antidepressant, anticonvulsant, antiparkinson's, and nootropic activities and neuroprotective activities [4-9]. Although both the plant has been studied for various CNS ailments, their potential was still unexplored.

This study will be focused on evaluation of the neuroleptic activity of the plant extract NS on various animal models of Psychosis.

MATERIALS AND METHODS:

Plants: Roots of N Jatamansi were obtained as a gift sample from National research institute of Ayurvedic Drug Development, Kolkata West Bengal. A sample specimen was kept safely in the herbarium sheet in the department of Pharmacognosy, Shri Vishnu college of Pharmacy, Bhimavaram for further reference.

Animals: Wistar Albino Rats of either sexes weighing 150-200 gm were obtained from Ghosh Entreprise Kolkata. They were housed in the Animal house of Shri Vishnu college of Pharmacy with the maintenance of 12 hrs day and night cycle. They were fed with normal pellet diet with sufficient water *ad libidum*. The study was approved by the Institutional animal ethics committee bearing the approval no 439/PO/01a/CPCSEA. The rats were acclimatized for 7days prior to the start of the study.

Plant Extracts: 500 gms of dried and powdered roots of the N. Jatamansi were extracted on Soxhlet extractor with ethanol for 5 days and after 5 days the extract were subjected to Rota evaporator to concentrate the extract. Later it was dried in vacuum to get the completely dried extracts.

Chemicals: Phencyclidine and amphetamine were obtained from the manufacturing company Sigma Aldrich, USA. Haloperidol sample was gifted by Cresent therapeutics limited, Solan, Himachal Pradesh.

Acute Toxicity Studies: Acute toxicity study was carried out for the ethanolic extracts of NJ following OECD guidelines. The Ethanolic extract was suspended in water with 2% w/v gum acacia with the dose of 5 mg kg^{-1} body weight was orally administered to overnight-fasted, healthy rats (n = 3). The animals were observed individually after dosing at least once during the first 30 min, periodically during the first 4 h, with special attention given during the first 24 h and daily thereafter for a total of 14 days. The acute toxicity study was repeated with doses of 50, 300 and 2000 mg kg^{-1} body weight.

Methods:

Amphetamine induced Stereotype in Rats [10]: Amphetamine is an indirect sympathomimetic agent. It induces licking, gnawing, grooming, sniffing (stereotype) in Rats which can be successfully prevented by classical neuroleptic agents. This test is predictive of antipsychotic drug, for D2 receptor antagonism. Two groups (n=6) of adult Wistar rats were taken weighing between 180 to 220gm and were treated with either test or the standard drug (Haloperidol) and then placed in individual cages. They were injected with d amphetamine (5mg/kg ip) after 30 mins. The onset of stereotypic behavior was evaluated at 30 mins interval for 3 hours. The reduction in mean stereotype score is indicative of antipsychotic effect.

Phencyclidine (PCP) Induced Bizarre pattern of locomotor activity [11]: Phencyclidine is a glutamate receptor antagonist. Administration of phencyclidine has been found to induce locomotor hyperactivity in rodents and is antagonized by antipsychotic drugs. Male Wistar rats weighing 150-200gm were housed in a chamber.

Animals were divided into groups (n=6), for test or the standard compound. 30 mins before the start of the experiment, the animals were administered with the extract or the standard drug. Phencyclidine (2mg/kg) was administered to the animals of both the groups just before the start of the experiment. Then the locomotor activity of the animals will be measured in photoactometer for a session lasting for 90 mins. Drugs antagonizing the phencyclidine induced activity are expected to act by some other receptor viz. Glutamatergic and Serotonergic rather than dopaminergic receptors.

Phencyclidine (PCP) Induced Social withdrawal test 11: This test helps to show the effectiveness of potential antipsychotic drugs against negative symptoms of schizophrenia. Phencyclidine decreases the time of social interaction in the rats. Naïve Male Wistar rats were housed in pairs for 10 days prior to the start of the experiment. During the test one, cage mate is removed and a new one is kept in the cage for 20 mins.

The amount of social interaction is measured as the total amount of time spent on various elements of interaction i.e. social exploration, and genital investigation. Phencyclidine will be administered 5 mins before the start of the experiment whereas the test or the standard drug will be given 30 mins before the experiment.

Conditioned Avoidance Response in rats [12] - Perhaps the oldest animal model to predict potential antipsychotic drug efficacy is the conditioned avoidance response (CAR). In the conditioned reinforcement model, experimental animals are trained to perform a certain response i.e. to avoid a mild shock. Trained avoidance responses may be active (pressing a lever, climbing a pole, or jumping out of a box).

Classical antipsychotic drugs reduce avoidance responding at doses that do not impair natural (untrained) escape. Three groups of rats (each having twenty rats) weighing 150-250 Gms were tested in this model for test drug (2 doses) or standard. 10 days of training period were carried out before the experiment, and a total of 20 sessions of training were imparted to each rat before the experiment. Test or the standard drugs were administered 30 mins before the start of the experiment.

Induction of catalepsy in Rats [10, 12]: Wistar rats weighing 180 to 200 gms each are randomly divided in three groups (test or standard). After an appropriate pretreatment time of the drug, each rat is tested for with respect to the right and left front paws which are first put on columns, first 3 cm and then 9 cm high. The cataleptic state was considered if the rat maintains the abnormal posture for 10 sec or more. The scoring was done according to the following

0- The rat moves normally when placed on a table.

1-Rats move only when touched or pushed.

1+1=2 – Rats placed on a table with front paws set alternately on a 3 cm high block fails to correct the posture in 10 secs, scored as 1 point for each paw, with a total of 2 for both paws.

1+1=2 – Rats placed on a table with front paws set alternately on a 9 cm high block fails to correct the posture in 10 secs, scored as 1 point for each paw, with a total of 2 for both paws.

This model predicts the extrapyramidal side effects of the test drug.

Estimation of Dopamine in different regions of the brain [13]: The following day of drug administration, the rats were decapitated and the brains were removed immediately according to the method described by Glowinski and Iversen [14]. The striatum and the frontal cortex regions were removed and were immediately frozen on dry ice and stored at -80°C. Striatal and frontal cortical tissues were sonicated in 0.1 M of perchloric acid (about 100 µl/mg tissue). The supernatant fluids were taken for measurements of levels of dopamine by HPLC. Briefly, 20 µl of supernatant fluid was isocratically eluted through a 4.6-mm C18 column containing paracetamol (100 mg/ml) as the internal standard with a mobile phase containing 50 mM ammonium phosphate pH 4.6, 25 mM hexane sulfonic acid pH 4.04, and 5% acetonitrile and detected by a UV spectrophotometer detector. The flow rate was 1 ml/ min. Concentration of DA was expressed as nanograms per gram of tissue.

Statistical Analysis: All the values will be expressed as mean ± SEM. Data analysis will be with the help One way ANOVA followed by Students ‘t’ test as when required.

Groupings of the animals:

Control Group – Animals of this group will receive 2% Gum acacia suspension.

Group II- Animals of this group will receive standard drug (Haloperidol)

Group III – Animals of this group will receive N Jatamansi (NJ) extract at a dose of 100mg/kg.

Group IV- Animals of this group will receive N. Jatamansi (NJ) extract at a dose of 200mg/kg

RESULTS:

Extraction: The amount of the extract obtained for N. Jatamansi was 15% of the initial material used.

Acute toxicity Studies: The ethanolic extract of plant was found to be safe upto the dose of 2000mg/kg body weight. The dose that was selected for the study was 100mg/kg and 200mg/kg body weight for the extract.

Amphetamine induced stereotypy in rats: Results from this study shows that all the stereotypic activities like sniffing rearing and licking were reduced significantly in the treatment groups($p<0.05$) compared to the control groups, but the degree of reduction varied differently among the treatment groups with no significant reduction with the lower dose of the extract**.** The standard drug haloperidol reduced sniffing, rearing and licking activity by 46%, 43% and 61% respectively. The ethanolic extract of NJ reduced sniffing, rearing and licking activity at higher dose by 17%, 32% and 21% respectively, whereas there was no reduction in any of the stereotypic activity with the lower dose (**Table 1**).

TABLE 1: INHIBITION OF AMPHETAMINE INDUCED STEREOTYPE

GROUPS	SNIFFING	REARING	LICKING
Control	17.62±2.25	7.72±1.17	4.71±0.97
Group II	9.56±3.75$^{\#}$	4.44±0.26$^{\#}$	1.84±1.17$^{\#}$
Group III	16.23±1.16	6.93±1.60	4.82±0.23
Group IV	14.67±2.20$^{\#}$	5.26±1.69$^{\#}$	3.74±0.16$^{\#}$

N=6: #= P<0.05 when compared with control

Phencycline induced bizarre pattern of locomotor activity: Results from this model are suggestive of no significant change in the locomotor activity for all the treatment groups compared to the control group. This result also suggests that the NJ extract did not alter the locomotor activity at any of their doses used (**Table 2**).

TABLE 2: PHENCYCLIDINE INDUCED BIZARRE PATTERN OF LOCOMOTOR ACTIVITY

GROUPS	LOCOMOTOR ACTIVITY SCORES
Control	302±7.28
Group II	305±6.23
Group III	297±12.70
Group IV	307±5.28

Phencyclidine (PCP) Induced Social withdrawal test: No animals from the test groups or the standard group altered the social exploration and the anogenital inspection activity compared with the control group significantly ($p>0.05$). This model is suggestive of the absence of negative symptoms alleviating property of all the test extracts and standards (**Table 3).**

TABLE 3: PHENCYCLIDINE INDUCED SOCIAL WITHDRAWL TEST

GROUPS	SOCIAL EXPLORATION	ANOGENITAL INSPECTION
Control	8±01.17	3±2.18
Group II	7 ±2.16	3±1.63
Group III	8±2.98	3±1.98
Group IV	9±2.29	3±1.69

Conditioned Avoidance Response in rats: All the groups significantly decreased the escape response compared to the control group ($p<0.05$). Group II reduced the escape response by almost 43%, Group III by 32% and group IV by 25% (**Table 4**).

TABLE 4: CONDITIONED AVOIDANCE RESPONSE IN RATS

GROUPS	NO OF TIMES ESCAPED
Control	16±1.97
Group II	9±1.23$^{\#}$
Group III	12±2.64$^{\#}$
Group IV	11±2.97$^{\#}$

N=6;#=p< 0.05 compared to control

Induction of catalepsy in Rats: All the treatment groups increased the mean cataleptic scores significantly ($p<0.05$) compared with the control group. However the increase in mean cataleptic score was increased by almost 100% in case of the test extract where as 350% in case of the standard drug haloperidol. However most the animals of the NJ treated groups corrected their stretched limb position within 10 seconds but they needed a touch or some kind of push for their movement to start.

There was no significant difference in cataleptic score among the different dose group of the test extracts (**Table 5**).

TABLE 5: INDUCTION OF CATALEPSY IN RATS

GROUPS	MEAN CATALEPTIC SCORES
Control	0
Group II	3.49.±057#
Group III	1.02±0.16#
Group IV	1.14±0.42#

N=6, #= Significant difference compared to control group

Estimation of Dopamine in different brain regions: The Dopamine estimation in the 2 regions of the brain suggested that the dopamine levels decreased in the frontal cortex for all the treatment groups including the standard but the decrease in dopamine concentration was more for the standard drug than the NJ extract when they were compared with the control ($p<0.05$) . However there were no significant changes in the striatum dopamine levels of the animals treated with the NJ extract when compared with the control, but there was a significant alteration in striatum dopamine levels in the standard group when compared with the control group (**Table 6**).

TABLE 6: ESTIMATION OF DOPAMINE IN DIFFERENT REGIONS OF THE BRAIN

GROUPS	Frontal cortex (ng/gm)	Corpus striatum (ng/gm)
Control	0.45±0.04	11.99±1.36
Group II	0.30±0.02#	9.42±1.52#
Group III	0.40±0.07#	11.11±1.96
Group IV	0.38±0.05#	12.24±1.77

N=6: #= P<0.05 when compared with control

DISCUSSION: Improving the effectiveness of antipsychotics appears to require proper and specific modulation of the various DA pathways. For instance, lessened extrapyramidal symptoms observed with newer agents are thought to be consequent to differential effects on the striatum and frontal cortex, respectively [15].

Haloperidol and Ethanolic extract of roots of NJ showed decrease in amphetamine induced stereotype compared to the control group. However the extent of decrease of the stereotypic activity for NJ was less as compared to the standard drug haloperidol. This kind of outcome was indicative of a possibility that the test extracts may be decreasing the Dopamine levels in the brain as is the case for the standard drug haloperidol [10].

Neither of the test extracts or the standard drug altered the phencyclidine induced increase in locomotor activity. Ineffectiveness of the extracts to show any effect on this model suggested that the extracts may not be acting on other neurotransmitter systems like glutamatergic or Serotonergic systems [11].

The extracts along with the standard drug did not have any impact on the phencyclidine induced social interaction test. This particular model was suggestive of the ineffectiveness of the test extracts to alleviate the negative symptoms of schizophrenia [11]. It is once again confirmed that haloperidol has no effect on the negative symptoms of schizophrenia.

The extracts as well as the standard drug reduced the conditioned avoidance response; however the magnitude of reduction was less for the test extract than the standard drug when they were compared with the control group. This kind of results for the standard and the test extracts again indicated the alleviating effects of positive symptoms of schizophrenia [12].

The induction of catalepsy once again pointed out the fact that both the extracts like the standard drug could be acting on the dopaminergic neurons of the brain [12]. Haloperidol is known to decrease the dopamine levels on various dopaminergic pathways of the brain which is the reason for extra pyramidal motor disorders. Further analysis of the data showed that there were no significant dose dependent effects for the NJ extract in decreasing the dopamine levels.

The reduction of the dopamine level in the frontal cortical regions of the brain was a kind of confirmatory result to establish the mode of antipsychotic action of the NJ extracts. This was a major finding of the study which was indicative and assertive of the mode of action of the NJ extracts. However the dopamine lowering activity for both the extracts was less, when compared to Haloperidol.

Nevertheless the unchanged dopamine level in the corpus striatum for both the extracts irrespective of the dose was also a significant observation for this study. This observation was pinpointing the fact that the test extract may not affect any kind of motor incoordination like that of the typical neuroleptic drugs [17].

However the extracts of NJ have shown the signs of catalepsy (in the animal model) although its magnitude was very less. Further studies are required in this context, although it can be argued that majority of the neuroleptic have considerable amount of sedative action and this can be mistaken for cataleptic activity as seen in the animal model when most of the animals moved on the table only when touched or pushed [17, 18].

Taking all the above facts into consideration, it may be safe to say that the ethanolic extract of the roots of N. Jatamansi decreases the dopamine levels in the frontal cortical regions of the brain. The dopamine lowering effect of the extracts were less than the standard drug but the encouraging fact was that the extracts did not alter the level of dopamine in the striatum region of the brain.

It was already established earlier that even the drugs having less affinity for the dopaminergic receptor than haloperidol, do have acceptable antipsychotic symptoms alleviating effect [19]. It's also well established that it is extremely essential for a molecule to have dopamine antagonistic activity to have any kind of neuroleptic activity [20]. Even the atypical antipsychotic drugs discovered later, need to have a certain degree of dopamine reducing activity apart from its interaction with other receptors viz. Serotonergic, Alpha adrenergic or Histaminergic [21, 22].

So it can be stated that by virtue of the dopamine lowering effect of the NJ extract in the frontal cortical region of the brain, they possess the antipsychotic effects.

Another significant outcome of this study was that the lower dose of the ethanolic NJ extract was not capable of decreasing the positive symptoms of schizophrenia as much as the higher dose (200mg/kg). So it seems that 200mg/kg body wt of ethanolic extract of NJ will be more effective in alleviating the positive symptoms of Schizophrenia.

CONCLUSION: The herbal extract used has shown promising effects in this study in reducing the positive symptoms of psychosis in rats by reducing the dopamine levels in the frontal cortical region of the brain. The extract can be further isolated to find out the active constituents responsible for this kind of activity which can also be major area of further research.

ACKNOWLEDGEMENT: I am grateful to the Principal, Director and the Management of Shri Vishnu college of Pharmacy for allowing me to utilize the research facilities of the college and providing me the necessities**.**

REFERENCES:

1. Hafner H and Heiden W: Epidemiology of Schizophrenia. The Canadian Journal of Psychiatry. 1997; 42:139-151.
2. Lieberman J A: Effectiveness of antipsychotic drugs in patients with chronic Schizophrenia. New Eng J of Med. 2005; 353 Suppl 12:1209-1217.
3. Roth BL, Tandra S, and Burgess LH: D4 receptor affinity does not distinguish between typical and atypical antipsychotic drugs. Psychopharmacology. 1995; 120 Suppl 3:332-337.
4. Vinutha JP: Acetyl cholinesterase inhibitory activity of methanolic and successive water extracts of *Nardostachys Jatamansi. Ind J Pharmacol* 2007; 23:127-131.
5. Rao VS, Rao A, and Karanth KS: Anticonvulsant and neurotoxicity profile of *Nardostachys Jatamansi* in rats. J Ethnopharmacology 2005; 102:351-6.
6. Prabhu V, Karanth KS, Rao A: Effects of *Nardostachys Jatamansi* on biogenic amines and inhibitory amino-acids in the rat-brain. Planta Med 1994; 60:114–117.
7. Ahmad M, Yousuf S, Khan Badruzzaman, Hoda Md N. Ahmad MA, Ishrat T , Agarwal AK and Islam F: Attenuation by *Nardostachys Jatamansi* of 6-hydroxydopamine-induced parkinsonism in rats: behavioral, neurochemical, and immunohistochemical studies. Pharmacol and Biochem Behav 2006 83:150-60.
8. Salim S, Ahmad M, Zafar KS, Ahmad AS, Islam F: Protective effect of *Nardostachys Jatamansi in rat cerebral ischemia.* Pharmacol and Biochem Behav 2003; 74:481-486.
9. Joshi H, Parle M: *Nardostachys Jatamansi* improves learning and memory in mice. J Med Food 2006; 9:113-8.
10. Kulkarni SK, Dandia PC: Ind J of Med. Res.1975; 63: 462-468.
11. 11.Corbett R, Camacho F, Woods S: Antipsychotic agents antagonise noncompetitive N methyl D aspartate antagonist induced behaviour.Psychopharmacology.1995; 1995:67-74
12. Matthysse S: Animal models in psychiatric research. Prog Brain Res.1986; 65:259-270.
13. Cleren C, Calingesan NY, Chen J: Celesterol protects against MPTP and 3 nitro propanoic acid induced neurotoxicity. J of Neurochem.2005; 94:995-1004.
14. Glowinski J and Iversen L: Regional studies of catecholamine in Rat brain. J of Neurochemistry.1966; 13: 655-669.
15. Baldessarini R: Drugs used in the treatment of

Psychiatric disorder. 10th Edition. Hardman J. Goodman Gilman's Pharmacological basis of therapeutics. Tennessee. Mc Graw Hill;2001:789-896

16. William P and Hollister L: Antipsychotic agents and Lithium.10th Edition. Bertam G. Katzung. Basic and Clinical Pharmacology. San Francisco. McGraw Hill;2007:457-454
17. Turner. A: Screening methods in Pharmacology. New York. Demic Press; 1965: 87-99.
18. Raymond B and Neumeyer J: Antipsychotics and Anxiolytic agents. 5th edition. David A Troy. Foyes principles of Medicinal Chemistry. Philadelphia. Lippincott; 2002: 408-433.
19. Rang HP, Dale MM, Ritter JM, and Flower RJ. Pharmacology7th Edition: Philadelphia: Elsevier; 2007:455-469
20. Anthony A, Martin R, Thurkauf A: Antipsychotic drugs. 6th Edition. Donald J Abraham. Burgers Medicinal chemistry and drug discovery. Virginia. Willey Interscience 2003:599-672
21. Green M: What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry. 1996; 153(1)*:* 321-330.
22. Goldberg TE, Gold JM, and Braff DL: Neuropsychological functioning and time-linked information processing is schizophrenia. Rev Psychiatry 1991; 10: 601-613.

How to cite this article:
Jash R, Choudary KA and Prasanth DSNBK: Evaluation of antipsychotic activity of ethanolic extract of *Nardostachys jatamansi* on Wistar albino rat. *Int J Pharm Sci Res* 2013: 4(7); 2730-2736. doi: 10.13040/IJPSR. 0975-8232.4(7).2730-36