

Effect of Hydro-Alcoholic Root Extract of *Nardostachys Jatamansi* on Haloperidol Induced Parkinsonism in Wistar Rats

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Summary

Nardostachys jatamansi roots were extracted with water(50%) & ethanol(50%) and analyzed for its anti-parkinsonism effect by measuring various neurological and behavioral parameters at the test dose of 250mg/kg and 500mg/kg body wt. Haloperidol 1mg/kg body weight was administered i.p in order to induce Parkinsonism. Parkinsonism caused by depletion of dopamine level in substantia nigra. Hydro-alcoholic root extract of *Nardostachys jatamansi* reversed the haloperidol induced Parkinsonism significantly ($p<0.01$), when compared to drugs, i.e. combination of L-dopa & Carbidopa (100mg+25mg/kg). From the above studies it could be predicted that the symptoms of Parkinsonism may be due to alteration in dopaminergic system, which play important role in the protection from Parkinsonism. The levels altered by haloperidol were restored significantly by the administration of Hydro-alcoholic root extract of *Nardostachys jatamansi*. The result of the study implies that *Nardostachys jatamansi* is a potent source to protect the brain from dopamine depletion level.

Key words: Haloperidol, *Nardostachys jatamansi*, Parkinsonism and L-dopa & Carbidopa,

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Introduction

Parkinson's disease (PD) is one of the major neurodegenerative disorders that affect the nerve cells in the part of the brain controlling muscle movement. Peoples with Parkinson's disease often experience trembling, muscles rigidity, difficult walking, problems with balance and slowed movements. These symptoms usually develop after the age 60.

The exact cause of this disease still remains a mystery this hampers the development of proper therapeutic interventions. Despite many approaches and efforts, to date no researchers have been successful in developing a cure or a modality to check the disease, and most of the therapies only provide functional relief. Evidence suggests that immense oxidative stress, free radical formation¹. Genetic susceptibility² programmed cell death³. And another unknown factor which might be endogenous (or) exogenous⁴. The neuropathology of the disease is based on depigmentation and cell loss in the dopaminergic nigrostriatal tract of the brain, with the corresponding decrease in the striatal dopamine (DA) concentration⁵.

Haloperidol is an antipsychotic or neuroleptic drug that produces parkinsonism in humans or catalepsy in animals. It has been attributed to a blockade of dopamine receptors⁶. Several brain regions appear to be involved in the expression of neuroleptic induced catalepsy.

Nardostachys jatamansi Jones DC belongs to the family *Valerianaceae* is an indigenous to the alpine Himalayan regions of India. The roots and rhizomes are useful part of plant. The sesquiterpens (such as Jatamansic acid, Jatamansone), lignans and neolignans have been reported to be present in the root of the plant^{7,8}. A new terpenoid ester, Nardostachysin (1) isolated from the rhizomes of the plants⁹. In Ayurveda, rhizomes of *N. Jatamansi* are used as a bitter tonic, stimulant, and antispasmodic and to treat hysteria, epilepsy and convulsions¹⁰. The decoction of the drug is also used in neurological disorders, insomnia and disorders of cardiovascular system¹¹. Ethanolic extract of *Nardostachys Jatamansi* is having anti-parkinsonism activity¹², anticonvulsant & neuro toxicity¹³. *Nardostachys Jatamansi* have reported the improved learning and memory activity in mice¹⁴, fungicidal activity¹⁵. The alcoholic extract of *Jatamansi* showed protective effect in thiacetamide induced liver damage in rats¹⁶. The extract of *Nardostachys Jatamansi* showed anti-oxidant and lipid peroxidation activity in doxorubicin-induced cardiac damage in rats¹⁷. The ethanolic extract of *N. Jatamansi* exhibited antidepressant activity only after a latency period of 7 days¹⁸.

Extract of *N. jatamansi* and *Rhus Sucedanea* shown significant effect on constrictor response of histamine, acetylcholine and serotonin on smooth muscles¹⁹. A traditional ayurvedic supplement containing *N. jatamansi* reported to have significant increase in sleep Latency²⁰. Acetone extract of *N. jatamansi* showed significant inhibition of Benzoyl Peroxide-Induced Cutaneous Oxidative stress, toxicity, and ear edema in mice²¹. The extract of *N. jatamansi* showed significant antiarrhythmic activity²². *N. jatamansi*; fumes and aerosols significantly ameliorated. The bronchial asthma in guinea pig induced by histamine²³. The extract of *N. jatamansi* produced sedative and depression action²⁴. 50% of ethanolic extract of curcuma longa (rhizome) and *N. jatamansi*; (whole plant) feeding elevated HDL cholesterol/total cholesterol ratio²⁵. The essential oils isolated from *N. jatamansi* cause the prolongation of hypotensive effect²⁶. These pharmacological properties of *N. jatamansi* prompted us to evaluate its efficacy in haloperidol induced parkinsonism.

Materials and Methods

Plant materials

Dried roots of *N. jatamansi* were purchased from an herbal market (Chennai, Tamilnadu, India). The specimen of the whole plant was identified and authenticated by Dr. P.Jayaraman, Director, Plant Anatomy Research Centre, Chennai. A specimen voucher was deposited at the department of pharmacology, C.L.Baid Metha college of Pharmacy, Chennai

Preparation of Hydro- Alcoholic extract of *N. jatamansi* root

The dried roots were grounded to small pieces and were dried under shade for 48hrs. The root pieces were made into coarse powder. The powder was passed through a 40 mesh sieve, to get uniform particle size. A weighed quantity of powder was subjected to continuous hot percolation in Soxhlet apparatus with water and alcohol in equal quantities at 50°C. The extract was evaporated under reduced pressure using Rota -flash evaporator until all the solvent had been removed. The yield of the extract was 12%w/w when compared to the dried starting material. The extract obtained was suspended in 1% v/v tween 80 for oral administration. The extract is subjected for its phytochemical screening.

Experimental animals

Inbred adult Wistar rats of either sex 80-90 days, weighing 150-200grms obtained from the animal house of C.L.Baid Metha college of Pharmacy were used for the study. The animals were maintained in a well ventilated room with 12hr light /dark cycle in standard poly propylene cages under control temp(26+₋1⁰C)and humidity(30-40%).They were fed with standard pellet diet obtained from poultry research station, Nandanam, Chennai and water ad libitum. The experimental protocol was approved by IAEC of C.P.C.S.E.A (ref no IAEC/08/14/CLBMCP/2005-2006, dated 20-12-2005).

TOXICITY STUDIES

Acute toxicity studies

Rats selected by random sampling technique were used in the study. Acute oral toxicity was performed as per OECD-423 guidelines²⁷. Three male Wistar rats weighing between 150-200grms were used for each dose. The starting doses level of aqueous root extract *N. Jatamansi* was selected as 5mg, 50mg, 500mg, 1000mg and 2000mg/kg/body wt, p.o. The drug was administered orally to rats, which were fasted overnight with water ad libitum before the administration of the drug. Body wt of the rat before and after treatment was noted. The animals were observed for toxic symptoms, such behavioral changes, locomotion, convulsions, and mortality for 72hrs.

Repeated oral toxicity studies

Repeated oral toxicity studies can be used to get additional information regarding the toxicity profile of a chemical. Repeated oral toxicity studies are defined as those studies where the chemical is administered to the animal for a period covering approximately 10% of the expected life of the animal. Usually, the dose levels are lower than for acute studies and allow chemicals to accumulate in the body before lethality occurs, if the chemical possess this ability²⁸.

Wistar rats of both sex 150-200grms were used for the study, were kept in a temperature-controlled environment ($26 \pm 1^{\circ}\text{C}$) with a 12 h light / dark cycle. Food and water were freely available and were recorded each 3 days. The animals were divided into one control group and one treated group. Each group consisting of six animals. The control group received tween 80 and each treated group received the Hydro alcoholic extract (1000 mg/kg body wt), by gavage for 28 days (once a day). The animals were weighed each 3 days. At the end of the experiment, blood was collected from the orbital sinus under ether anesthesia for biochemical and hematological analysis. After the blood collection, the animals were sacrificed by cervical displacement and selected organs (brain, liver, heart, testis, ovaries, pancreas, lungs and kidney). Small pieces of brain, lungs, liver, heart, pancreas, kidney, ovaries and testis were collected in 10% formalin solution for preparation of sections by using microtome. The histopathological studies were carried out by a standard method²⁹.

PHARMACOLOGICAL STUDIES

Measurement of Parkinsonism by (Block Method)³⁰

The animal were divided into four groups each consisting of 6 animals

Group 1: Control receiving 1% tween 80(1ml/100gm)

Group 2: Haloperidol (1mg/kg)

Group 3: Hydro Alcoholic extract of *N. Jatamansi* (250mg/kg) suspended in 1%v/v tween 80 for 15 days and haloperidol (1mg/kg)

Group 4: Hydro Alcoholic extract of *N. Jatamansi* (500mg/kg) suspended in 1%v/v tween 80 for 15 days and haloperidol (1mg/kg)

Group 5: Standard L-Dopa & Carbidopa (100mg+25mg/kg i.p) 1 hr prior to the Challenge with haloperidol.

Severity of Parkinsonism was measured every 30 mints there after up to total duration for 3 hrs. Catalepsy of an individual rat was measured in a stepwise manner by a scoring method.

Step-1

The rat was taken out of the home cage and placed on a table. If the rat failed to move when touched gently on the back (or) pushed, score of 0.5 was assigned.

Step-II

The front paws of the rats were placed alternately on a 3cm high block. If the rat failed to correct the posture with in 15 seconds, a score of 0.5 for each paw was added to the score of step 1.

Step III

The front paws of the rat placed alternately on a 9cm high block .if the rat failed to correct the posture with in 15sec, a score of 1 for each paw was added to the scores of step I, step II. Thus, for an animal, the highest score was 3.5(cut off score) and that reflects in total Parkinsonism.

Behavioral assessment (Metal bar test)³¹ the effect of the test and standard drug on behavioral assessment in haloperidol-administered rats was studied by the following method.

The animal were divided into four groups each consisting of 6 animals.

Group 1: Control receiving 1% tween 80(1ml/100gm)

Group 2: Haloperidol (1mg/kg)

Group 3: Hydro alcoholic extract of *N. Jatamansi* (250mg/kg) suspended in 1%v/v tween 80 for 15 days and haloperidol (1mg/kg)

Group 4: Hydro Alcoholic extract of *N. Jatamansi* (500mg/kg) suspended in 1%v/v tween 80 for 15 days and haloperidol (1mg/kg)

Group 5: Standard L-Dopa & Carbidopa (100mg+25mg/kg i.p) 1 hr prior to the challenge with haloperidol.

Procedure

A cataleptic behavior was measured with a high bar test method. Catalepsy score was measured for 4 hours at one hour intervals after haloperidol administration by gently placing both the fore paw of the rat over a metal bar (diameter 2-5mm suspended 6cm above the tabletop). The intensity of catalepsy assessed by counting the time in seconds until the rat brought both fore pass down to the tabletop, with a maximum cutoff time of 3 minute. Finally, scores at different time points (0, 60,120,180 & 240 minutes after haloperidol injection) were added and expressed as cumulative catalepsy score for comparison purpose.

Results

The hydro-alcoholic extract showed the presence of steroids, sterols, glycosides, carbohydrates, alkaloids, tannins, terpenes, gums and mucilage. Flavonoids, proteins, saponins and phenols were not present. The acute oral toxicity was done according to the OECD guide lines 423(acute toxicity class method).There was no considerable change in the body wt before and after treatment of the experiment and no sign of toxicity were observed (Table 1). Repeated oral toxicity carried out by administration of the hydro-alcoholic extract of *N. Jatamansi* at a dose of 1000mg/ kg/ body wt, p.o for 28 days. No significant changes in body wt observed. The hydro-alcoholic extract of *N. Jatamansi* treated rats did not show any significant changes in hematological parameters (like hemoglobin RBC, WBC, Neutrophils, Monocytes, Esinophiles and lymphocytes value) when compared with the normal control animal (Table 2). Histopathological examinations of internal organs like liver, testis, ovaries, pancreas, lungs, kidney, heart, pancreas and brain, did not shows any changes in their normal architecture suggesting no damage caused by hydro-alcoholic extract of *N. Jatamansi* Haloperidol induced Parkinsonism significantly ($p<0.01$) at a dose of 1 mg/kg /ip. The Haloperidol induced Parkinsonism was decreased by the treatment of Hydro-alcoholic root extract of *N. Jatamansi*, L-Dopa & Carbidopa. The maximal decrease in Parkinsonism was observed in group V animals. The Hydro-alcoholic extract of *N. Jatamansi* at a dose of 500mg/kg has more significant effect ($p<0.01$) than 250mg/kg in the reversal of haloperidol induced Parkinsonism. The combination of L-Dopa & Carbidopa at a dose of (100mg+25mg/kg i.p) also shown significant effect ($p<0.01$) in the reversal of haloperidol induced Parkinsonism which is assessed by block method (table3) and metal bar test (table 4)

Table 1 Acute toxicity study of aqueous extract of *N. Jatamansi* in rats (OECD guideline 423)

S.no	Drug treatment	Dose	Average body wt of the animal in gms		Signs of toxicity	Effect observed	Death
			Before treatment (1 st day)	After treatment (14 th day)			
1	Hydro alcoholic extract of <i>NJ</i>	5mg/kg	160	175	No sings of toxicity	No effect	Nil
2	Hydro alcoholic extract of <i>NJ</i>	50mg/kg	175	180	No sings of toxicity	No effect	Nil
3	Hydro alcoholic extract of <i>NJ</i>	500mg/kg	180	192	No sings of toxicity	No effect	Nil
4	Hydro alcoholic extract of <i>NJ</i>	1000mg/kg	175	190	No sings of toxicity	No effect	Nil
5	Hydro alcoholic extract of <i>NJ</i>	2000mg/kg	160	170	No sings of toxicity	No effect	Nil

Table 2 Repeated oral toxicity study of ANJ for 28 days in rats. Hematological parameters

S.no	Groups	Hb (gm %)	RBC (million s/cmm)	WBC (per cmm)	Differential count			
					Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Esinophils (%)
1.	Control	16.62 ± 0.06256 ^{ns}	6.297 ± 0.16222 ^{ns}	10487 ± 274.08 ^{ns}	55.33 ± 1.406 ^{ns}	40.17 ± 0.8333 ^{ns}	2.333 ± 0.6148 ^{ns}	2.167 ± 0.5426 ^{ns}
2.	Test	17.63 ± 0.08028 ^{ns}	7.075 ± 0.1420 ^{ns}	9710 ± 246.8 ^{ns}	50.17 ± 1.167 ^{ns}	43.83 ± 1.447 ^{ns}	2.667 ± 0.6146	3.500 ± 0.6708 ^{ns}

Values are expressed in mean ± SEM. Each group consists of 6 mice
 Statistical significance test for comparison was done by student's t, test
 ns; Non significance .

Table- 3: Effect of aqueous extract of *N. Jatamansi* on Haloperidol induced Parkinsonism. A. Block method

S.No	Drug treatment	30 min	60 min	90 min	120 min	150 min	180 min
1.	Tween 80	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2.	Haloperidol treated	1.5±0.0 ^{a**}	2.833±0.206 ^{a**}	3.33±0.16 ^{a**}	3.5±0.0 ^{a**}	3.166±0.20 ^{a**}	3.08±0.270 ^{a**}
3.	N.Jatamansi(250mg/kg)+ Haloperidol	1.000±0.1826 ^{bns}	2.03±0.346 ^{bns}	1.563±0.1054 ^{b*}	1.354±0.1054 ^{b**}	0.8245±0.2234 ^{b**}	0.5000±0.2367 ^{b**}
3.	N.Jatamansi(500mg/kg)+ Haloperidol	0.6523±0.147 ^{bns}	1.5±0.1532 ^{bns}	1.333±0.1023 ^{b*}	1.082±0.0235 ^{b**}	0.5000±0.2214 ^{b**}	0.00±0.00 ^{b**}
4.	L-Dopa +carbidopa Haloperidol	0.5833±0.8333 ^{b**}	1.654±0.1127 ^{b**}	1.25±0.1655 ^{b**}	1.02±0.1324 ^{b**}	0.500±0.2367 ^{b**}	0.0±0.0 ^{b**}

Values are mean ± SEM of four samples of six observations. Statistical significant test for comparison was done by ANOVA, followed Dennett's test.

a-Group I and Group II, b-Group II Vs Group III, Group IV

*p< 0.05; **P<0.01; Vs non significant.

Table – 4: Effect of aqueous extract of *N. Jatamansi* on Haloperidol induced Parkinsonism.**B. Metal Bar test**

S.No	Drug treatment	0 min	60 min	120 min	180 min	240 min
1.	Tween 80	5.500±1.088	6.167±1.138	8.167±0.7923	4.667±0.5578	9.667±0.9545
2.	Haloperidol treated	8.833±0.8724 ^{ans}	145.3±3.333 ^{a**}	180±3.651 ^{a**}	180.0±5.625 ^{a**}	150.7±5.270 ^{a**}
3.	N.Jatamansi(250mg/kg)+ Haloperidol	5.500±0.8851 ^{bns}	25.50±3.354 ^{b**}	20.50±2.141 ^{b**}	18.00±1.826 ^{b**}	15.00±1.826 ^{b**}
3.	N.Jatamansi(500mg/kg)+ Haloperidol	4.312±0.432 ^{bns}	23.10±1.4236 ^{b**}	19.146±3.472 ^{b**}	17.365±1.689 ^{b**}	13.265±0.265 ^{b**}
4.	L-Dopa & carbidopa +Haloperidol	7.167±0.6540 ^{bns}	20.00±4.830 ^{b**}	17.83±3.468 ^{b**}	15.00±3.109 ^{b**}	10.17±2.007 ^{b**}

Values are mean ± SEM of four samples of six observations. Statistical significant test for comparison was done by ANOVA, followed Dennett's test.

a-Group I and Group II, b-Group II Vs Group III, Group IV

*P< 0.05; **P<0.01; Vs non significant.

Discussion

The present study demonstrates the Anti-parkinsonism effect of Hydro-alcoholic root extract of *N. Jatamansi* in haloperidol model of Parkinson's disease in rats. Haloperidol induced Parkinsonism in rats is used to screen the drug for their anti-parkinsonism effect.. Hydro-alcoholic root extract of *N. Jatamansi* at the dose of 500mg/kg, p.o, exhibited a more pharmacological effect than 250mg/kg when compared to L-Dopa & Carbidopa (100mg+25mg/kg i.p). Dopamine depletion is considered as a cardinal feature in causing a Parkinsonism in humans (or) in animal models. The enhancement of dopamine content by *N. Jatamansi* treatment might have restored the alteration in loco motor activity, exploratory behavior³².Alcoholic extract of *N. Jatamansi* having dopamine enhancing property³³ and antioxidant potential property^{34,32} might have afforded protection in the haloperidol induced Parkinson's disease .Several studies have reported on exacerbation of neuroleptic induced catalepsy by enhanced serotonergic neurotransmission in the CNS³⁵.The two main dopamine pathway in the brain are the mesolimbic pathway and the nigrostriated pathway. It can be hypothesized from this study that hydro alcoholic extract of *N. Jatamansi* inhibits the symptoms of haloperidol induced Parkinsonism in rats. The mechanisms by which the amelioration takes place may be attributed to one (or) more pharmacological /biochemical mechanism Viz. Hydro-alcoholic root extract of *N. Jatamansi* may enhance the bioavailability of circulating dopamine by up regulation of dopaminergic signaling.

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