

Evaluation of toxicological and antioxidant potential of *Nardostachys jatamansi* in reversing haloperidol-induced catalepsy in rats

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Abstract: An aqueous root extract from *Nardostachys jatamansi* was investigated for its antioxidant and anticataleptic effects in the haloperidol-induced catalepsy rat model of the disease by measuring various behavioral and biochemical parameters. Catalepsy was induced by administration of haloperidol (1 mg/kg, *ip*) in male albino rats. A significant ($P < 0.01$) reduction in the cataleptic scores were observed in all the drug-treated groups as compared to the haloperidol-treated group; with maximum reduction observed in the *Nardostachys jatamansi* (250 and 500 mg/kg body weight) administered group. To estimate biochemical parameters: the generation of thiobarbituric acid reactive substances (TBARS); reduced glutathione (GSH) content and glutathione-dependent enzymes; catalase; and superoxide dismutase (SOD), in the brain were assessed. Haloperidol administration increased generation of TBARS and significantly reduced GSH, which were restored to near normal level with the *Nardostachys jatamansi* treatment. Catalase and SOD levels were also increased to normal levels, having been reduced significantly by haloperidol administration. Our findings of behavioral studies and biochemical estimations show that *Nardostachys jatamansi* reversed the haloperidol-induced catalepsy in rats. We conclude that the antioxidant potential has contributed to the reduction in the oxidative stress and catalepsy induced by haloperidol administration.

Keywords: *Nardostachys jatamansi*, antioxidant, toxicological, haloperidol and catalepsy

Introduction

The brain is deficient in oxidative defense mechanisms and hence is at a greater risk of damage mediated by reactive oxygen species (ROS), resulting in molecular and cellular dysfunction.¹ ROS can damage virtually any biological molecule in its vicinity including; DNA, essential proteins, and membrane lipids.² Parkinson's disease is a neurodegenerative disease characterized by the selective loss of dopamine neurons of the *substantia nigra pars compacta*. The events, which trigger and/or mediate the loss of nigral dopamine neurons, remains unclear. Neuroleptic-induced catalepsy has long been used as an animal model for screening drugs for parkinsonism.³ Catalepsy is defined as the failure to correct an externally imposed posture. A condition characterized by inactivity, decreased responsiveness to stimuli, and a tendency to maintain an immobile posture. The limbs tend to remain in whatever position they are placed (waxy flexibility). Catalepsy may be associated with psychotic disorders (eg, schizophrenia, catatonia), nervous system drug toxicity, and other conditions. The catalepsy test is widely used to evaluate motor effects of drugs that act on the extrapyramidal system.⁴ Evidence suggests that immense oxidative stress, free radical formation,⁵ genetic susceptibility,⁶ and programmed cell death⁷ are the main causes for neurodegeneration associated with Parkinson's and other

related diseases. 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) concentration.⁸ Besides, dopamine receptor blockade and catecholamine depletion, other neurochemical hypotheses have been proposed for the development of catalepsy such as striatonigral GABAergic (gamma-aminobutyric acid), cholinergic, glutamate, and serotonergic.^{9–11} Haloperidol is an antipsychotic drug which is used in the treatment of schizophrenia and other affective disorders. It blocks dopaminergic action in the nigrostriatal pathway leading to a high frequency of extrapyramidal motor side effects.¹² In animal models, haloperidol induces a behavioral state known as catalepsy in which the animals are unable to correct externally imposed postures.¹³ The use of haloperidol has been associated with an increased level of oxidative stress in the brain.¹⁴ This evidence suggests a possible role for antioxidants in the treatment of haloperidol-induced catalepsy. The brain is made up of 70% lipid and any kind of stress is usually manifested by lipid peroxidative damage.¹⁵ The extent of this damage can be used to evaluate the degree of cellular harm. Stress-induced lipid peroxidative damage in the brain can be quantified by either determining the amount of peroxidative products or the rates of enzyme-catalyzed reactions neutralizing free radical intermediates such as superoxide dismutase (SOD). SOD is a primary, natural, and free radical scavenging and antioxidant enzyme in the body. The estimation of the activity of such antioxidant enzymes such as SOD, catalase, or glutathione peroxidase, can be used to assess the therapeutic effects of different antioxidant agents.¹⁶

Nardostachys jatamansi (*N. jatamansi*) (*Valerianaceae*) is indigenous to the Himalayan regions of India. In Ayurveda, roots and rhizomes of *N. jatamansi* are used to treat hysteria, epilepsy, and convulsions.¹⁷ The decoction of the drug is also used in neurological disorders, insomnia and disorders of cardiovascular system.¹⁸ The sesquiterpenes (Jatamansic acid, Jatamansone), lignans and neolignans are reported to be present in the roots of this plant.^{19,20} Rhizomes are reported to contain a terpenoid ester, Nardostachysin I.²¹ To date much research has been undertaken to evaluate the drug to treat various neurological and cardiovascular disorders in various animal models and is widely used in ayurvedic formulations. It is reported to possess antidepressant activity,²² anticonvulsant activity,²³ antiarrhythmic activity,²⁴ and possess antioxidant, and lipid peroxidation activity in doxorubicin-induced cardiac damage in rats.²⁵ It is also reported to improve learning and memory in mice²⁶ and to enhance biogenic amine activity.²⁷ An acetone extract of *N. jatamansi*

has shown significant inhibition of benzoyl peroxide-induced cutaneous oxidative stress, toxicity, and ear edema in mice.²⁸ It has also been reported to possess protective activity in 6-hydroxydopamine-induced, parkinsonism in rats.²⁹ These pharmacological properties of *N. jatamansi* prompted us to evaluate its efficacy in haloperidol-induced parkinsonism.

Materials and methods

Plant material

Dried roots of *N. jatamansi* were purchased from a herbal market (Chennai, Tamil Nadu, India) and authenticated by Dr P Jayaraman, Director of the Plant Anatomy Research Centre, Chennai, India. A specimen voucher was deposited at the department of Pharmacology, C L Baid Metha College of Pharmacy, Chennai, India.

Preparation of aqueous extract of *N. jatamansi*

The dried roots were coarsely powdered and extracted with distilled water for 48 hours by cold maceration at room temperature before being filtered. The filtrate was concentrated in rotary vacuum evaporator. Percentage yield was calculated with respect to the dried material and screened for its phytoconstituents. The extract obtained was suspended in 1% v/v Tween 80 for oral administration.

HPTLC profile

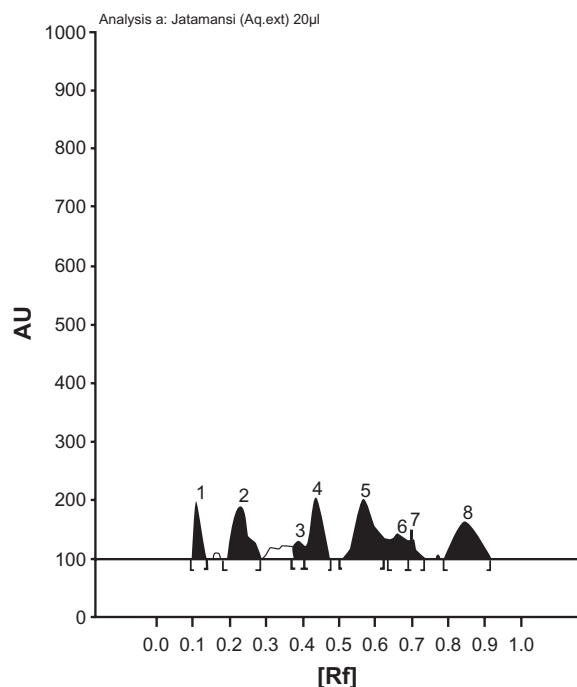
Chromatography was performed on high performance thin layer chromatography (HPTLC) plates coated with 0.25 mm layer of silica gel 60 F254 (Merck, München, Germany). Before using, the plates were washed with methanol and activated at 110°C for 5 minutes. Samples were applied as 4 mm wide bands and 6 mm apart by using a Linomat IV sample applicator (Camag Muttentz, Basel, Switzerland) equipped with 100 µL syringe. A constant application rate of 5 µL/second was used. The mobile phase was petroleum ether-acetone (3:1 v/v) and chromatograms were monitored at 254 nm (Figure 1).

Experimental animals

Inbred adult Wistar rats of either sex, weighing 150–200 gm were obtained from the animal house of the C L Baid Metha College of Pharmacy, India. The animals were maintained in a well-ventilated room with a 12-hour light/dark cycle in standard polypropylene cages under controlled temperature (26 ± 1°C) and humidity (30%–40%). They were fed with a standard pellet diet obtained from Poultry Research Station, Nandanam, Chennai, India. Water was supplied to the

SPECTRUM :M: JATAMAN2 Anrutanjan Ltd 14/JUL/2006 12:54

Method Scan Integration Calibration Spectrum Data End HELP



Wave length: 254 m

Track: 1. noise level: 0.137AU. raw data file: JATAMAN2

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Track 1. Analysis a: Jatamansi (Aq.ext) 20µl

Peak #	Start		Max		[%]	End		Area	
	Rf	H	Rf	H		Rf	H	F	[%]
1	0.10	2.8	0.11	95.2	17.07	0.14	1.0	1181.7	8.28
2	0.19	0.2	0.23	87.1	15.62	0.28	4.2	2579.9	18.09
3	0.37	18.8	0.39	29.6	5.32	0.41	16.6	461.9	3.24
4	0.41	16.6	0.44	101.3	16.17	0.48	1.3	2245.1	15.74
5	0.50	0.5	0.57	98.2	17.60	0.62	33.7	3589.0	25.16
6	0.64	31.0	0.66	38.9	6.97	0.69	26.7	1141.3	8.00
7	0.69	26.7	0.70	48.2	8.64	0.74	1.2	443.8	3.11
8	0.79	3.6	0.89	59.1	10.60	0.92	0.0	2622.4	18.38

Total height = 557.7

Total area = 14265.1

Figure 1 HPTLC chromatogram of aqueous extract of *N. jatamansi*.**Abbreviation:** HPTLC, high performance thin layer chromatography.

animals ad libitum. Experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of C.P.C.S.E.A with ref no. IAEC/08/14/CLBMCP/2005–2006, dated 12–20–2005.

Toxicity studies

Acute toxicity studies

Rats selected by a random sampling technique were used in the study. Acute oral toxicity was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines.³⁰ Three male Wistar rats weighing between 150–200 g were used for each dose. The dose levels of 5 mg, 50 mg, 500 mg, 1000 mg, 2000 mg, and 5000 mg/kg/body weight, per os were selected. The lethal dose (LD)-50 value of

the extract was determined. The drug was administered orally to rats, which were fasted overnight with water ad libitum before the administration of the drug. The body weight of the rat was noted before and after treatment. The animals were observed for toxic symptoms, such behavioral changes, locomotion, convulsions, and mortality for 72 hours.

Repeated oral toxicity studies

In repeated oral toxicity studies drug extracts are 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 possesses this ability.³¹ Wistar rats of both sexes, 150–200 g

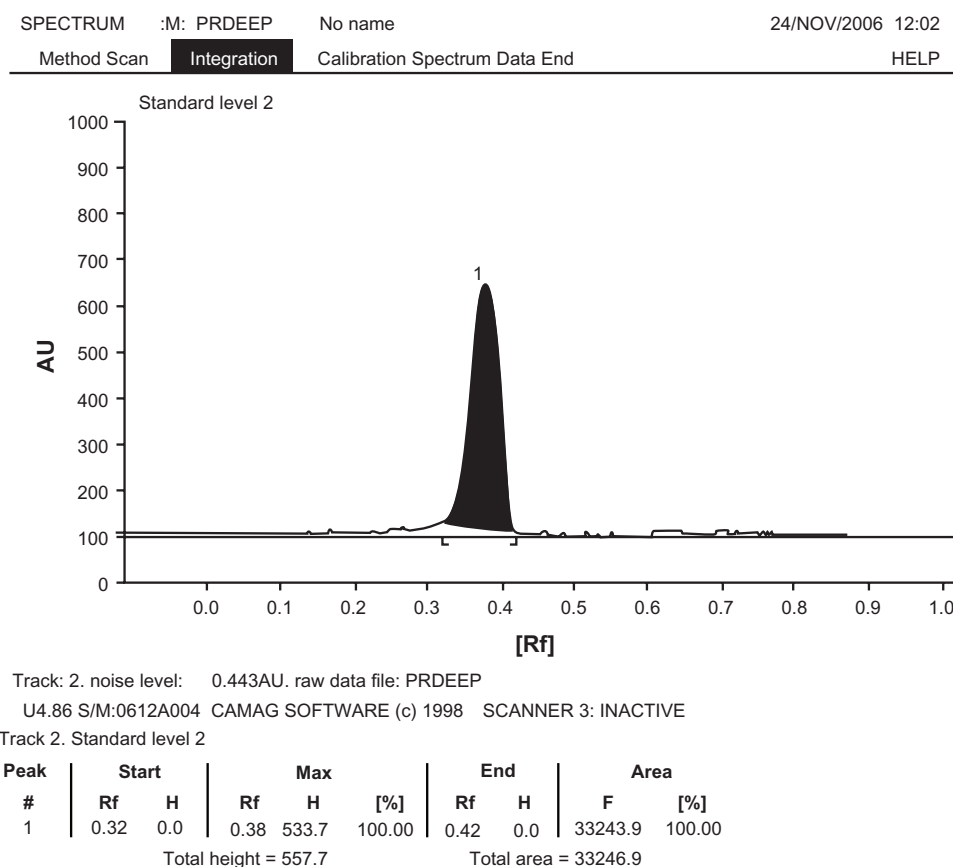


Figure 2 HPTLC chromatogram of standard (Reference compound) Jatamansone.
Abbreviation: HPTLC, high performance thin layer chromatography.

were used for the study and kept in a temperature-controlled environment ($26^{\circ}\text{C} \pm 1^{\circ}\text{C}$) with a 12-hour light/dark cycle. Food and water were freely available and were recorded each at 3-day intervals. 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 aqueous extract (1000 mg/kg body weight), by gavage once a day for 28 days. The animals were each weighed every 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, lungs, liver, heart, pancreas, kidney, and testis) were removed and collected in 10% formalin solution in readiness for preparation of sections. The histopathological studies were carried following the method of Mukherjee.³²

Experimental design

Adult male Wistar rats (180–250 g) were divided into six groups each containing six animals. Group I received the vehicle 1% Tween 80 solution and served as the control. Group II received

haloperidol alone and served as the cataleptic control without any drug treatment. Group III received a combination of L-dopa and carbidopa (100 mg + 25 mg/kg by intraperitoneal administration) and served as positive control. Groups IV, V, and VI received *N. jatamansi* at a dose of 100, 250, 500 mg/kg body weight, respectively. Thirty minutes after the administration of these drugs, catalepsy was induced by the intraperitoneal administration of haloperidol at a dose of 1 mg/kg body weight in normal saline. This procedure was repeated for 15 days. All the behavioral studies were performed at room temperature in a calm room without any external interference. The severity of catalepsy was measured every 30 minutes thereafter for a total duration for 3 hours. Catalepsy of an individual rat was measured in a stepwise manner using a scoring method described below. After 15 days of behavioral studies, animals were sacrificed by cervical dislocation and the whole brain was immediately dissected out and washed in ice-cold saline to remove all traces of blood. The brains were weighed and a 10% tissue homogenate was prepared in 0.025 M Tris–HCl buffer at pH 7.5 and used to measure the activities of thiobarbituric acid reactive substances (TBARS). Enzyme activity was assayed in 10% brain homogenates prepared in 0.2 M phosphate buffer, pH 8.0.

Table 1 Acute toxicity study

Drug treatment	Dose	Average body weight of the animal in grams		Signs of toxicity	Effect observed	Death
		Before treatment (1st day)	After treatment (14th day)			
Aqueous extract of <i>N. jatamansi</i>	5 mg/kg	169	175	No signs of toxicity	No effect	Nil
Aqueous extract of <i>N. jatamansi</i>	50 mg/kg	160	169	No signs of toxicity	No effect	Nil
Aqueous extract of <i>N. jatamansi</i>	500 mg/kg	167	174	No signs of toxicity	No effect	Nil
Aqueous extract of <i>N. jatamansi</i>	1000 mg/kg	173	179	No signs of toxicity	No effect	Nil
Aqueous extract of <i>N. jatamansi</i>	2000 mg/kg	155	164	No signs of toxicity	No effect	Nil
Aqueous extract of <i>N. jatamansi</i>	5000 mg/kg	182	195	No signs of toxicity	No effect	Nil

Notes: Body weight and signs of toxicity observed following treatment of aqueous extract of *N. jatamansi* in rats for 14 days.

Behavioral studies

Measurement of catalepsy by block method³³

This scoring method followed is in three steps. **Step 1:** The rat was taken out of the home cage and placed on a table. If the rat failed to move when touched or pushed gently on the back a score of 0.5 was assigned. **Step II:** The front paws of the rats were placed alternately on a 3-cm high block. If the rat failed to correct the posture within 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 were placed alternately on a 9-cm high block, if the rat failed to correct the posture within 15 seconds a score of 1 for each paw was added to the scores of steps I and II. Thus, the highest score for any animal was 3.5 (cut off score) and that reflects total catalepsy (Table 3).

Behavioral assessment by metal bar test

Behavioral assessment in haloperidol-induced cataleptic rats was studied by the method of Kulkarni.³⁴ 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 forepaws of the rat over a metal bar (diameter 2–5 mm suspended 6 cm above the table top). The intensity of catalepsy assessed by counting the time in seconds until the rat brought both forepaws down to the tabletop, with a maximum cutoff time of 3 minutes. Finally, scores at different time points (0, 60, 120, 180 and 240 minutes after haloperidol injection) were added and expressed as a cumulative catalepsy score for comparison purposes (Table 4).

Biochemical studies

Estimation of lipid peroxidation products

Lipid peroxidation was estimated colorimetrically in brain tissue by quantifying TBARS according to the method of Niehaus and Samuelson.³⁵ In brief; for the estimation of TBARS the supernatant of the tissue homogenate was treated with tertiary butanol–trichloroacetic acid–hydrochloric acid, (TBA–TCA–HCl) reagent and mixed thoroughly. The mixture was kept in boiling water bath for 15 minutes. After cooling, the tubes were centrifuged for 10 minutes and the supernatant

Table 2 Repeated oral toxicity studies

Groups	Hb (gm %)	RBC (millions/mm ³)	WBC (per mm ³)	Differential count			
				Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Control	14.62 ±	5.297 ±	7487 ±	50.33 ±	45.17 ±	2.333 ±	2.167 ±
	0.06256 ns	0.16222 ns	274.08 ns	1.406 ns	0.8333 ns	0.6148 ns	0.5426 ns
Test	14.63 ±	5.075 ±	7710 ±	52.17 ±	41.83 ±	2.667 ±	3.500 ±
	0.08028 ns	0.1420 ns	246.8 ns	1.167 ns	1.447 ns	0.6146 ns	0.6708 ns

Notes: Values are expressed in mean ± SEM. Each group consists of 6 rats. Statistical significance test for comparison was done by Student's t-test.

Abbreviations: ns, not significant; Hb, hemoglobin; WBC, white blood corpuscles; RBC, red blood corpuscles.

Table 3 Effect of aqueous extract of *N. jatamansi* on haloperidol-induced catalepsy by block method

Drug treatment	30 min	60 min	90 min	120 min	150 min	180 min
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
Haloperidol	2.00 ± 0.224*	2.833 ± 0.211a**	3.5 ± 0.0a**	3.5 ± 0.0a**	3.167 ± 0.211a**	3.333 ± 0.167a**
<i>N. jatamansi</i> (100 mg) + haloperidol	1.85 ± 0.457*	2.792 ± 0.322*	2.612 ± 0.563*	2.356 ± 0.043b*	2.114 ± 0.63b*	1.923 ± 0.673b**
<i>N. jatamansi</i> (250 mg) + haloperidol	1.500 ± 0.342*	2.500 ± 0.259*	2.167 ± 0.211*	1.833 ± 0.211b**	1.417 ± 0.0833b**	1.02 ± 0.823b**
<i>N. jatamansi</i> (500 mg) + haloperidol	1.00 ± 0.213*	2.080 ± 0.326*	1.526 ± 0.243**	1.287 ± 0.0833b**	1.236 ± 0.056b**	0.5000 ± 0.224b**
L-dopa and carbidopa + haloperidol	0.5833 ± 0.833*	1.667 ± 0.117**	1.333 ± 0.105**	1.250 ± 0.112b**	1.13 ± 0.042b**	0.5 ± 0.0b**

Notes: Values are mean ± SEM (n = 6). Statistical analysis by one-way ANOVA, followed by Dunnett's multiple comparison test.

*P < 0.05, **P < 0.01.

Abbreviations: SEM, standard error of mean; Min, minutes.

taken for measurement. The developed color was read at 535 nm using a UV spectrophotometer (Hitachi 912) against a reagent blank and expressed as mM per 100 g tissue.

Estimation of antioxidants

Catalase (CAT) was assayed colorimetrically at 620 nm and was expressed as micromoles of H₂O₂ consumed per minute per mg of protein; using the method described by Sinha.³⁶ The reaction mixture (1.5 mL, volume) contained 1.0 mL of 0.01 M pH 7 phosphate buffer, 0.1 mL of tissue homogenate and 0.4 mL of 2 M H₂O₂. The reaction was stopped by the addition of 2 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in the ratio of 1:3). The assay for SOD was based on SOD mediated inhibition of the reduction of nitroblue tetrazolium to blue formazan by superoxide anions as described by Beauchamp and Fridovich.³⁷ The total protein present in the homogenate was estimated following the method described by Lowry.³⁸ Units of SOD activity determined were expressed in terms of milligrams of total protein (TP). Reduced glutathione (GSH) was determined

by the method of Ellman.³⁹ One mL of supernatant was treated with 0.5 mL of Ellman's reagent and 3 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. The activity of GSH was expressed as nM GSH formed/g tissue.

Statistical analysis

Each group of rats assigned to a specific drug treatment each group consisted of 6–9 animals. All the values are expressed as mean ± standard error of mean (SEM). The data were analyzed by analysis of variance (anova) followed by Tukey test. The criterion for statistical significance was P < 0.05.

Results

The phytochemical analysis of an aqueous root extract of *N. Jatamansi* revealed the presence of sterols, carbohydrates, terpenes, phenols, gums and mucilage. The HPTLC fingerprint of the aqueous extract showed the presence of eight spots (R_f values 0.11, 0.23, 0.39, 0.44, 0.57, 0.66, 0.70 and 0.84) (Figure 1) at a wavelength of 254 nm. The standard

Table 4 Effect of aqueous extract of *N. jatamansi* on Haloperidol induced catalepsy by metal bar test

Drug treatment	0 min	60 min	120 min	180 min	240 min
Tween 20	8.500 ± 1.088	7.167 ± 1.138	5.167 ± 0.792	5.667 ± 0.558	5.667 ± 0.955
Haloperidol treated	5.833 ± 0.872	138.3 ± 3.333**	165 ± 3.651**	165.0 ± 5.625**	161.7 ± 5.270**
<i>N. jatamansi</i> (100 mg) + Haloperidol	6.832 ± 0.056	102 ± 2.348*	94.5 ± 4.387*	72.3 ± 2.567*	51.2 ± 3.246*
<i>N. jatamansi</i> (250 mg) + Haloperidol	8.167 ± 0.654	65.00 ± 4.830**	58.83 ± 3.468**	48.00 ± 3.109**	23.33 ± 2.472**
<i>N. jatamansi</i> (500 mg) + Haloperidol	5.643 ± 0.423	43.42 ± 3.147**	36.23 ± 4.123**	28.3 ± 2.148**	18.24 ± 0.185**
L-Dopa and carbidopa + Haloperidol	7.500 ± 0.8851	32.50 ± 3.354**	27.50 ± 2.141**	23.00 ± 1.826**	15.00 ± 1.826**

Notes: Values are mean ± SEM (n = 6). Statistical analysis by one-way ANOVA, followed by Dunnett's multiple comparison test.

*P < 0.05, **P < 0.01.

Abbreviations: SEM, standard error of mean; Min, minutes.

reference compound, Jatamansone, had the R_f value of 0.39 (Figure 2), hence it is confirmed that the aqueous extract contains terpenoids. The acute oral toxicity was undertaken according to the OECD guidelines 423 (acute toxicity class method). There was no considerable change in body weight either before or after experimental treatment and no signs of toxicity were observed (Table 1). The LD50 test of the aqueous extract was found to be greater than 5000 mg/kg body weight after oral administration. Repeated oral toxicity was carried out by administration of the extract at a dose of 1000 mg/kg body weight per os for 28 days. The extract treated rats did not show any significant changes in hematological parameters (hemoglobin red blood corpuscles [RBC], white blood corpuscles [WBC], neutrophils, monocytes, eosinophils and lymphocytes) when compared with the normal control animals (Table 2). Histopathological examinations of the internal organs, ie, liver, kidney and brain, did not show any changes in their normal architecture, suggesting the safety of the drug (Figure 3) as the oral administration of the drug to normal rats had no significant

effect. Based on these findings a dose of 100, 250, and 500 mg/kg bodyweight were selected for the study. The cataleptic scores of the present study are given in Table 3 and 4, assessed by block method and metal bar test, respectively. Haloperidol induced catalepsy significantly ($P < 0.01$) at a dose of 1 mg/kg (intraperitoneal administration). Significant reversal in haloperidol-induced catalepsy was observed with the administration of *N. jatamansi* aqueous extract and combination of L-dopa and carbidopa. The maximal decrease ($P < 0.01$) in catalepsy was observed in the group receiving aqueous extract of *N. Jatamansi* at a dose of 250 mg/kg. However, there was no pronounced reduction in the cataleptic scores at a dose of 500 mg/kg, but almost similar results were observed as observed at a dose of 250 mg/kg body weight.

The levels of lipid peroxidation products and antioxidants in the brains of haloperidol, drug-treated and control groups are shown in Table 5. The haloperidol-treated rats showed a significant increase ($P < 0.01$) in TBARS and there was also a significant reduction ($P < 0.01$) in SOD, CAT, and GSH in the brain tissue. Oral administration of the extract

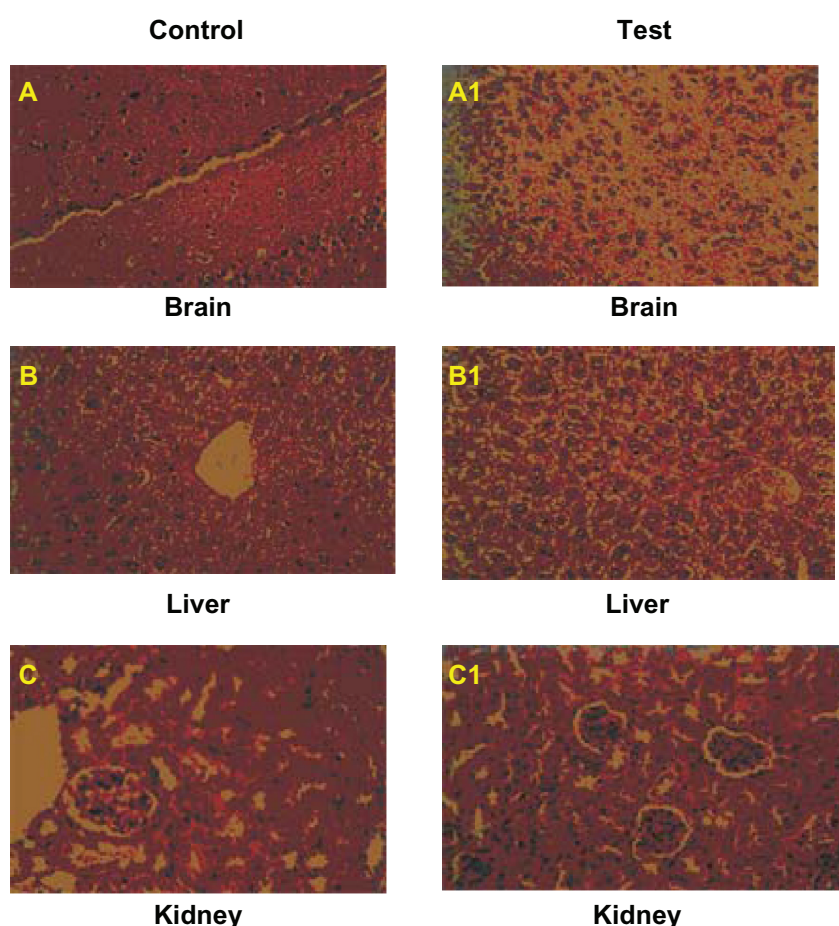


Figure 3 Histopathological slides of Brain (A control, A1 test), Liver (B control, B1 test) and Kidney (C control, C1 test), Repeated oral toxicity study of *Nardostachys jatamansi* treatment for 28 days in rats showed no pathological changes.

Table 5 Effect of *N. jatamansi* on TBARS, SOD, CAT, and GSH levels in normal and catalepsy-induced rat brain

Group	TBARS (mM/100 g tissue)	SOD (U ^a)	Catalase (U ^b)	GSH (mg/100 g tissue)
Control	1.13 ± 0.08	7.83 ± 0.70	2.99 ± 0.27	11.53 ± 0.50
Haloperidol treated	1.99 ± 0.12***	4.58 ± 0.31***	0.75 ± 0.05***	5.82 ± 0.33***
L-dopa and carbidopa + Haloperidol	1.22 ± 0.05***	6.42 ± 0.28***	2.19 ± 0.22***	9.15 ± 0.28***
<i>N. jatamansi</i> (100 mg) + Haloperidol	1.31 ± 0.43*	5.78 ± 0.45**	1.95 ± 0.20*	8.48 ± 0.51*
<i>N. jatamansi</i> (250 mg) + Haloperidol	1.15 ± 0.06***	6.81 ± 0.50***	2.49 ± 0.29**	9.98 ± 0.46***
<i>N. jatamansi</i> (500 mg) + Haloperidol	1.18 ± 0.21***	6.89 ± 0.38***	2.43 ± 0.23**	9.94 ± 0.38***

Notes: Values are mean ± SEM of six samples of six observations. Statistical significant test for comparison was done by ANOVA, followed Dunnett's test. ^aAmount of enzyme required to inhibit 50% of NBT reduction per mg protein. ^bMicromoles of H₂O₂ consumed per min per mg protein.

Abbreviations: SEM, standard error of mean; TBARS, thiobarbituric acid reactive substances; GSH, reduced glutathione; CAT, catalase; SOD, superoxide dismutase.

along with haloperidol administration significantly restored ($P < 0.01$) the peroxides and antioxidant levels to near normal in the brains of the test animals. For all the parameters studied, *N. jatamansi* extract administered at doses of 250 and 500 mg/kg bodyweight showed significant effects. L-dopa and carbidopa also showed a significant effect in all the parameters studied in rats.

Discussion

The central nervous system is especially vulnerable to free radical damage because of the brain's high oxygen consumption, its abundant lipid content, and the relative paucity of antioxidant enzymes as compared with other tissues.⁴⁰ Evidence also indicates that ROS may stimulate extracellular release of excitatory amino acids.⁴¹ Glutamate is the major excitatory amino acid in the brain which acts through various types of ionotropic receptors, the most significant being *N*-methyl *D*-aspartate (NMDA) receptors. There seems to be a bidirectional relationship between the ROS production and the release of excitatory amino acids.⁴² Free radicals generated in the brain are also reported to influence gene expression, subsequently effecting apoptosis and neuronal death.⁴³ In the brain, an array of cellular defense systems exists to counterbalance the ROS. These include enzymatic and nonenzymatic antioxidants that lower the concentration of free radical species and repair oxidative cellular damage. The brain is known to synthesize molecules like glutathione and NADPH. Glutathione functions as a major antioxidant in tissue defense against free radicals in the brain. However, the concentration of glutathione is, relatively, in lesser quantities in the brain as compared to the other organs of the body.⁴⁰ The natural antioxidant system present in brain can be in form of enzymes like catalase, peroxidase, superoxide dismutase or low molecular weight antioxidants (ascorbic and lipoic

acids, carotenoids or indirectly acting chelating agents).⁴³ Free radical scavengers or antioxidants function as biological bodyguards for essential molecules by either neutralizing reactive species before they mutilate a molecule or they repair damage that has been inflicted.

The present study demonstrates the antioxidant effects of an aqueous root extract of *N. Jatamansi* in haloperidol-induced, cataleptic oxidative stress in rats. The induction of free radicals in mammals by haloperidol is well established. Previous studies have shown that dopamine receptors in the striatum are involved in neuroleptic-induced catalepsy. It has been demonstrated that the cataleptic effects of haloperidol are apparently mediated by dopamine receptors localized postsynaptically on strial neurons.¹³ It is also well established that the administration of haloperidol leads to an increase in the oxidative stress in the brain tissue.¹⁴ The increase in SOD observed in the present study supports the this concept. Superoxide formation is a major factor in oxygen toxicity and the superoxide dismutase enzyme constitutes an essential defense against it. Under normal conditions, decreased activity of antioxidant enzymes, such as SOD, glutathione peroxidase and catalase, in the brain leads to the accumulation of oxidative free radicals resulting in degenerative effects.⁴⁴ An increase in these enzymes under normal conditions would represent increased antioxidant activity and a protective mechanism in neuronal tissue, thus, constituting the first line of defense against oxidative stress in our body. However, in the presence of a free radical-quenching agent, the induction of the antioxidant enzymes is minimized. So, any overall decrease in cataleptic scores and SOD activity in the drug treated groups indicates the ability of the drug extract to combat oxidative stress in brain tissue and reduce the severity of haloperidol-induced catalepsy. The altered balance of the antioxidant enzymes caused by the decrease in CAT, SOD, GSH activities may be responsible for the inadequacy of the antioxidant defenses in

combating ROS mediated damage. The decreased activities of CAT and SOD may be a response to increased production of H_2O_2 and O_2 by the autooxidation.⁴⁵ It has been suggested that these enzymes play an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides. Treatment with *N. jatamansi* extract increased the activity of these enzymes by quenching the free radicals. Previously *N. jatamansi* has been reported to be a well known antioxidant and the ethanol extract of *N. jatamansi* is reported to possess potent antioxidant activity that scavenges free radicals generated after the induction of catalepsy.²⁷ Significantly lower levels of lipid peroxides in the brains of the drug-treated group and increased activities of enzymatic and nonenzymatic antioxidants in the brain suggests that the extract reduces oxidative stress. In previous studies *N. jatamansi* has been reported to have antilipid peroxidative and protective effect in rat cerebral ischemia.^{46,47} *N. jatamansi* has also been reported to enhance biogenic amine activity,⁴⁸ decrease the level of dopamine and its metabolites and increasing the number of dopaminergic D2 receptors in striatum.²⁹ Such evidence supports our study and indicates that the extract of *N. jatamansi* inhibits the symptoms of haloperidol-induced catalepsy in rats. The action by which the amelioration takes place may be attributed to one (or) more pharmacological/biochemical mechanisms. To conclude, the brain exhibits numerous morphological and functional alterations during oxidative stress, a factor implicated in the pathogenesis of many CNS disorders. Treatment of such neuronal disorders with *N. jatamansi* plant extract significantly decreases lipid peroxidation and significantly increases the antioxidants in the brain. The findings of this study suggest the possible antioxidant role of *N. jatamansi* in overcoming behavioral and neurochemical changes during oxidative stress. Since the catalepsy test has predictive value regarding extrapyramidal effects, the possibility of pharmacological interactions between haloperidol and *N. jatamansi* extract should be further investigated in clinical studies.

Disclosures

The authors report no conflicts of interest relevant to this research.

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