Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Suppl 4, 2012 ISSN - 0974-2441

Research Article

PHYTOCHEMICAL, TOXICOLOGICAL EVALUATION AND ANTICATALEPTIC ACTIVITY OF *NARDOSTACHYS JATAMANSI*

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Received: 17 September 2012, Revised and Accepted: 16 October 2012

ABSTRACT

Hydro alcoholic root extract from *Nardostachys jatamansi* was investigated for its antioxidant and anti cataleptic effects in the haloperidol-induced catalepsy rat model of the disease by measuring behavioral, biochemical parameters and neurotransmitter levels. Catalepsy was induced by administration of haloperidol (1 mg/kg, i.p) in male wistar 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* (500 mg/kg body weight) administered group. 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. Haloperidol administration decreased the dopamine levels significantly, which were restored to near normal levels with the *Nardostachys jatamansi* treatment. Our findings of behavioral studies and biochemical estimations show that *Nardostachys jatamansi* reversed the haloperidol-induced catalepsy in rats.

Keywords: *Nardostachys jatamansi*, Haloperidol, Antioxidant, Dopamine and Catalepsy.

INTRODUCTION

Catalepsy induced by neuroleptics may be due to a blockade of dopaminergic neurotransmission in the striatum.[1-3] Haloperidol is an antipsychotic drug which is used in the treatment of schizophrenia and other affective disorders. It blocks dopaminergic action in the nigrostraital pathway leading to a high frequency of extrapyramidal motor side effects.[4] In animal models, haloperidol induces a behavioral state known as catalepsy in which the animals are unable to correct externally imposed postures.[5] The use of haloperidol has been associated with an increased level of oxidative stress in the brain.[6] This evidence suggests a possible role of antioxidants in the treatment of haloperidol-induced catalepsy. Neuroleptic-induced catalepsy has long been used as an animal model for screening drugs for Parkinsonism.[7] The events, which trigger and/or mediate the loss of nigral dopamine neurons, remains unclear. 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). The catalepsy test is widely used to evaluate motor effects of drugs that act on the extrapyramidal system.[8] Evidence suggests that immense oxidative stress, free radical formation,[9] genetic susceptibility,[10] and programmed cell death[11] 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 nigrostraital tract of the brain, with the corresponding decrease in the striatal dopamine (DA) concentration.[12] 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 serotonoergic.[13-15] The brain is made up of 70% lipid and any kind of stress is usually manifested by lipid peroxidative damage.[16] 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.[17]

Nardostachys jatamansi (Valirenaceae) is indigenous to the Himalayan regions of India. In Ayurveda, roots and rhizomes of *Nardostachys jatamansi are* used to treat hysteria, epilepsy, and convulsions.[18] The decoction of the drug is also used in neurological disorders, insomnia and disorders of cardiovascular system.[19] 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,[20] anticonvulsant activity, [21] antiarrhythmic activity, [22] and possess antioxidant, and lipid peroxidation activity in doxorubicin-induced cardiac damage in rats. [23] these pharmacological properties of *Nardostachys jatamansi* prompted us to evaluate its efficacy in haloperidol-induced catalepsy.

MATERIALS AND METHODS

Plant materials

Dried roots of *Nardostachys jatamansi* were purchased from an herbal Market (Hyderabad, Andhra Pradesh, India.) and authenticated by Dr.K.Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati. A specimen voucher was deposited at the Department of Pharmacology, Nizam Institute of Pharmacy, India.

Preparation of Hydro alcoholic extract of *Nardostachys jatamansi*:

The dried roots were coarsely powdered and weighed quantity of powder was subjected to continuous hot percolation in soxh let apparatus with 1:1 ratio of ethanol and water at 65-70 ^{o}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 14% w/w. when compared to the dried starting material. The extract obtained was suspended in 1% v/v Tween 80 for oral administration.

HPTLC analysis

i) Preparation of extract of the sample drug:

200mg Hydro alcoholic root extract of *Nardostachys jatamansi*, was taken and dissolved in the corresponding solvents and make up to 10ml and applied on the TLC plate.

ii) Development and determination of the solvent system:

Sample applied	:	Sample drug solution of about 5µl.
Solvent system	:	petroleumether: acetone (3:1)
Scanning wavelength	:	254nm

The sample was spotted with the help of automatic TLC applicator system of the DESAGA Sarstedt Gruppe on the Merck precoated aluminum sheets of silica gel 60 F_{254}. After trying to various solvent system with variable volume ratio, the suitable solvent system was selected as stated above in its proportional ratio and developed in the Twin through chamber of TLC to the maximum height of the plate so that it can be able to separate the components on the polar phase of silica gel and that of mobile phase of solvent system. The components get separated by the principle of adsorption, having differential migration rates of individual component towards the phases.

iii) Development of HPTLC technique:

After the development, TLC plate was removed and dried completely and detected with the suitable detection system. UV cabinet system was used for detection of spots. Further it was scanned with the densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 254nm. A corresponding densitogram was obtained, in which peaks are appeared for the corresponding spots, being detected in the densitometer while scanning, and the peaks area under the curve corresponds to the concentration of the component in the sample for the concentration applied on the TLC plate.

Experimental animals

Inbred adult Wistar rats of either sex, weighing 150–200 gm were obtained from the animal house of the Nizam Institute of Pharmacy, Deshmukhi, Ramoji Film City, Hyderabad. Before and during the experiment, the animals were maintained in a well-ventilated room with a 12-hour light/dark cycle in standard poly propylene cages under controlled temperature (26 ± 1•C) and humidity (30%–40%). They were fed with a standard pellet diet obtained from Gold Moher, Lipton India Ltd, Hyderabad and water *ad libitum* throughout the experimental period. All animal experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee) with registration number. (1330/AC/10/CPCSEA).

Acute toxicity

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.[24] Three male Wistar rats weighing between150–200g were used for each dose. The dose levels of 5mg, 50mg, 500mg, 1000mg, 2000mg, and 5000 mg/kg/body weight, per oral dose 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 as behavioral changes, locomotion, convulsions and mortality for first 24 hours and there after up to 14 days continuously.

Experimental Design

Adult male Wistar rats (150–200gm) 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 negative control without any drug treatment, group III received combination of L-dopa and carbidopa (100 mg ± 25 mg/kg by intraperitoneal administration) and served as positive control and Groups IV, V, VI received *Nardostachys jatamansi* at doses of 100, 250, 500 mg/kg body weight, respectively for 15 days. Catalepsy was induced by the intraperitoneal administration of haloperidol at a dose of 1mg/kg body weight in normal saline. All the behavioral studies were performed at room temperature in a calm room without any external interference. After the 15 days, 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.

Behavioral studies

Measurement of catalepsy by block method[25] This scoring method was followed 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 (cutoff score) and that reflects total catalepsy.

Behavioral assessment by metal bar test

Behavioral assessment in haloperidol-induced cataleptic rats was studied by the method of Kulkarni.[26] 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 5).

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.[27] In brief; for the estimation of TBARS the supernatant of the tissue homogenate was treated with tertiary butanol-trichloroaceticacid-hydrochloricacid, (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 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 100g tissue.

Estimation of antioxidants

Catalase (CAT) was assayed colorimetrically at 620 nm and was expressed as micromoles of H_2O_2 consumed per minute per mg of protein; using the method described by Sinha.[28] 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 H_2O_2. 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 nitro blue tetrazolium to blue formazan by superoxide anions as described by Beauchamp and Fridovich.[29] The total protein present in the homogenate was estimated following the method described by Lowry.[30] Units of SOD activity determined were expressed in terms of milligrams of total protein (TP). Reduced glutathione (GSH) wasdetermined by the method of Ellman. [31] 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.

Estimation of Dopamine Levels

Procedure

The method of DeVito and Wagner, [32] as described by Zafar [33] was used for the estimation of DA. The striatum (20% w/v) was sonicated in 0.4 N perchloric acid containing 100 ng/mL of the internal standard 3,4-dihydroxybenzylamine, followed by centrifugation at 15,000 x g for 10 min at 4ºC and the filtrate was injected manually through a 20-µL loop over the ODS-C_{18} column coupled with HPLC/Electrochemical detector (Waters, Milford, MA, USA) for separation and quantification. The mobile phase consisted of 0.1 M potassium phosphate (pH 4.0), 10% methanol, and 1.0 mM heptane sulfonic acid. Samples were separated on an ODS-C_{18} column using a flow rate of 1.0 mL/min. The concentrations of DA was calculated using a standard curve generated by determining ratio between three known amounts of the amine or its metabolites and a constant amount of internal standard and represented as ng/mg of tissue.

Statistical analysis

Each group of rats assigned to a specific drug treatment each group consisted of 6 animals. All the values are expressed as mean ± standard error of mean (SEM). The data were analyzed by analysis of variance (ANOVA) followed by Dunnet's multiple comparison test.

RESULTS

The phytochemical analysis of hydro alcoholic extract of *Nardostachys jatamansi* revealed the presence of carbohydrates, Alkaloids, steroids, sterols, tannins, flavonoids, gums and mucilage, glycosides and terpenes. The HPTLC fingerprint of the hydro alcoholic extract of *Nardostachys jatamansi* showed the presence of eight spots (Rf values 0.03, 0.35, 0.48, 0.57, 0.74, 0.81, 0.93 and 0.98) (Table 1, Figure 1) at a wavelength of 254 nm.

Table 1: peak list of haenj chromatogram at 254 nm in solvent system of petroleum ether: acetone (3:1)

Peak no.	Y-pos	Area	Area (%)	Height	Rf
1	11.0	3651.53	48.2	1324.32	0.03
2	29.8	466.59	6.2	114.91	0.35
3	37.3	658.57	8.7	139.15	0.48
4	42.4	578.35	7.6	160.25	0.57
5	52.6	600.82	7.9	133.22	0.74
6	56.7	456.17	6.0	133.09	0.81
7	63.9	795.88	10.5	167.00	0.93
8	66.9	371.52	4.9	154.02	0.98

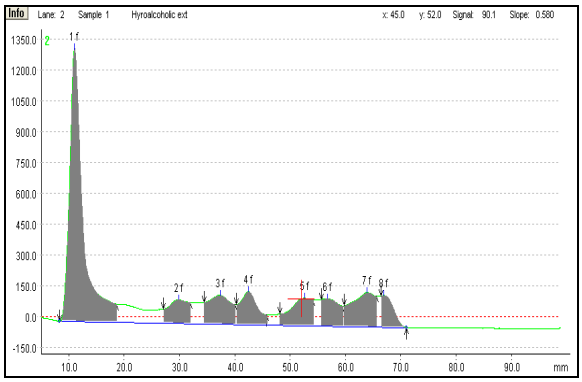

Fig 1: densitogram of haenj at 254 nm developed in solvent system of petroleum ether: acetone (3:1)

Table 2: weight and signs of toxicity observed following treatment of haenj in rats for 14 days

S.No	Drug treatment	Dose	Average body Weight of animal in gms Before treatment (1st day)	After treatment (14th day)	Signs of toxicity	Effect observed	Death
1	Vehicle control	10ml/kg	170	178	No signs of toxicity	No effect	Nil
2	HAENJ	5g/kg	160	175	No signs of toxicity	No effect	Nil
3	HAENJ	50g/kg	175	180	No signs of toxicity	No effect	Nil
4	HAENJ	100g/kg	180	192	No signs of toxicity	No effect	Nil
5	HAENJ	1000g/kg	175	190	No signs of toxicity	No effect	Nil
6	HAENJ	2000g/kg	160	170	No signs of toxicity	No effect	Nil
7	HAENJ	5000g/kg	190	203	No signs of toxicity	No effect	Nil

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 2). The LD_{50} test of the hydro alcoholic 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 oral 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 3).

Histopathological examinations of the internal organs, i.e, brain, heart, kidney, liver, lung and pancreas, did not shown any changes in their normal architecture, suggesting the safety of the drug (Figure 2) 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.

Table 3: haematological parameters observed in rats followed by the treatment of haenj for 28 days

Group	Drug treatment	HB	RBC	WBC	Differential count Neutrophils	Lymphocytes	Monocytes	Esinophyls
1.	Vehicle control	16.62 ± 0.0625 ns	6.297 ± 0.1622 ns	10487 ± 274.08 ns	55.33 ± 1.406 ns	40.17 ± 0.833 ns	2.333 ± 0.6148 ns	2.167 ± 0.5426 ns
2.	HAENJ (1000mg/kg)	17.63 ± 0.0802 ns	7.075 ± 0.1420 ns	9710± 246.8 ns	50.17 ±1.167 ns	43.83 ± 1.447 ns	2.667 ± 0.6146 ns	3.500 ± 0.6708 ns

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

The cataleptic scores of the present study are given in Table 4, 5, and Figure 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 hydro alcoholic extract of *Nardostachys jatamansi* and combination of l-dopa and carbidopa. The maximal decrease ($P < 0.01$) in catalepsy was observed in the group receiving hydro alcoholic extract of *Nardostachys jatamansi* at a dose of 500 mg/kg.

Table 4: effect of haenj on haloperidol induced catalepsy by block method

Group	Drug treatment	30 min	60 min	90 min	120 min	150 min	180 min
1	Vehicle control	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 (1mg/kg)	1.5 ± 0.0**	2.833 ± 0.206**	3.33 ± 0.16**	3.5 ± 0.0**	3.166 ± 0.20**	3.08 ± 0.270**
3	L-dopa+carbidopa (100mg+25mg/kg)+ haloperidol (1mg/kg)	0.5833 ± 0.8333**	1.654 ± 0.1127**	1.25 ± 0.1655**	1.02 ± 0.1324**	0.500 ± 0.2367**	0.0 ± 0.0**
4	HAENJ(100mg/kg) +haloperidol (1mg/kg)	2.32 ± 0.436	2.0 ± 0.152	1.852 ± 0.432*	1.654 ± 0.126**	1.125 ± 0.3264**	0.732 ± 0.3215**
5	HAEN(250mg/kg)+ Haloperidol (1mg/kg)	1.000 ± 0.1826	2.03 ± 0.346	1.563 ± 0.1054*	1.354 ± 0.1054**	0.8245 ± 0.2234**	0.5000 ± 0.2367**
6	HAENJ(500 mg/kg)+ haloperidol (1mg/kg)	0.6523 ± 0.147	1.5 ± 0.1532	1.333 ± 0.1023*	1.082 ± 0.0235**	0.5000 ± 0.2214**	0.00 ± 0.00**

Values were mean ± SEM (n=6). Statistical analysis by One-way ANOVA, followed by Dunnett's multiple comparison tests. *p< 0.05. **P<0.01.

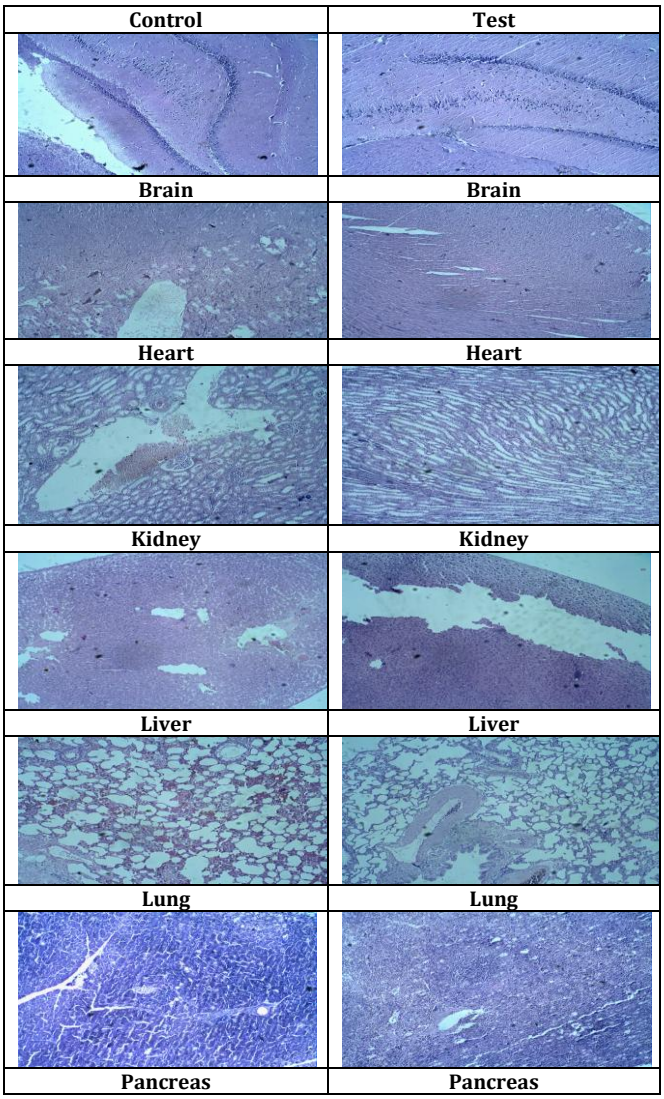

Fig 2: histopathology of organs of the rats treated with haenj for 28 days

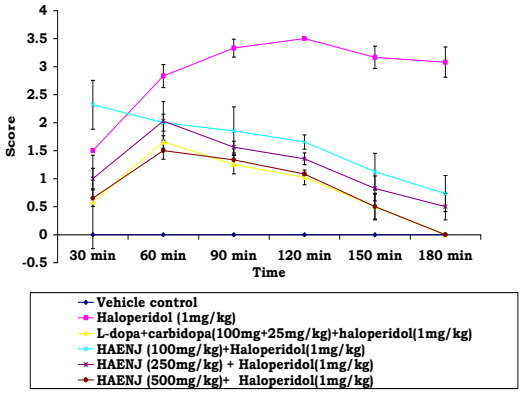

Fig 3: effect of haenj on haloperidol induced catalepsy by block method.

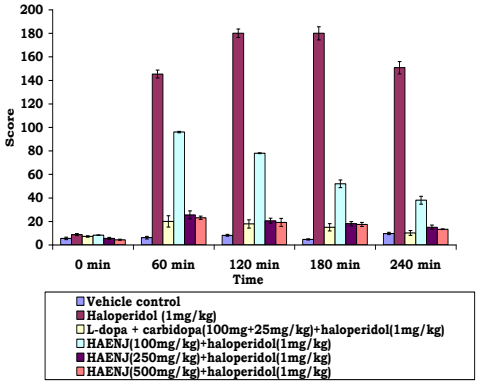

Fig 4: effect of haenj on behavioral assessment in Haloperidoladministered rats by metal bar test.

The levels of lipid peroxidation products and antioxidants in the brains of haloperidol, drug-treated and control groups are shown in Table 6 and Figure 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 along with haloperidol administration significantly restored (P < 0.01) the peroxides and antioxidant levels to near normal in the brains of the test animals.

TABLE 5: EFFECT OF HAENJ ON BEHAVIORAL ASSESSMENT IN HALOPERIDOL ADMINISTERED RATS BY METAL BAR TEST

Group	Drug treatment	0 min	60 min	120 min	180 min	240 min
1	Vehicle control	5.500± 1.088	6.167 ± 1.138	8.167± 0.7923	4.667± 0.5578	9.667± 0.9545
2	Haloperidol (1mg/kg)	8.833± 0.8724	145.3± 3.333**	180 ± 3.651**	180.0± 5.625**	150.7± 5.270**
3	L-dopa + carbidopa (100 mg+25 mg/kg)+ haloperidol (1mg/kg)	7.167± 0.6540	20.00± 4.830**	17.83± 3.468**	15.00± 3.109**	10.17± 2.007**
4	HAENJ (100 mg/kg) +haloperidol (1mg/kg)	8.264± 0.0326	96± 0.564*	78 ± 0.326*	52± 3.256*	38± 3.324*
5	HAENJ (250mg/kg)+ haloperidol (1mg/kg)	5.500± 0.8851	25.50± 3. 354**	20.50± 2.141**	18.00± 1.826**	15.00± 1.826**
6	HAENJ (500mg/kg)+ haloperidol (1mg/kg)	4.312± 0.432	23.10± 1.4236**	19.146± 3.472**	17.365± 1.689**	13.265± 0.265**

Values were mean ± SEM (n=6). Statistical analysis by One-way ANOVA, followed by Dunnett's multiple comparison tests. *p< 0.05. **P<0.01.

TABLE 6: EFFECT OF HAENJ ON TBARS, SOD, CAT AND GSH LEVELS IN HALOPERIDOL ADMINISTERED RAT BRAIN.

Group	Drug treatment	TBARS (mM/100g tissue)	SOD (U^A)	Catalase (U^B)	GSH (mg/ 100g tissue)
1	Vehicle control	1.13 ± 0.08	7.83 ± 0.70	2.99 ± 0.27	11.53 ± 0.50
2	Haloperidol (1mg/kg)	1.99 ± 0.12***	4.58 ± 0.31***	0.75 ± 0.05***	5.82 ± 0.33***
3	L-dopa + carbidopa(100mg + 25mg/kg) + haloperidol(1mg/kg)	1.22 ± 0.05***	6.42 ± 0.28***	2.19 ± 0.22***	9.15 ± 0.28***
4	HAENJ (100 mg/kg) + haloperidol (1mg/kg)	1.52 ± 0.52*	5.24 ± 0.86**	1.72 ± 0.36*	8.2 ± 0.28*
5	HAENJ (250 mg/kg) + haloperidol(1mg/kg)	1.25 ± 0.42***	6.4 ± 0.48***	2.18 ± 0.54**	9.84 ± 0.36***
6	HAENJ (500 mg/kg)+ haloperidol (1mg/kg)	1.20 ± 0.36***	6.78 ± 0.53***	2.25.± 0.41**	9.92 ± 0.58***

Values were mean ± SEM of six samples of six observations. Statistical significant test for comparison was done by ANOVA, followed Dunnett's test. **A**-Amount of enzyme required to inhibit 50% of NBT reduction per mg protein. **B**-Micromoles of H_20_2 consumed per min per mg protein.

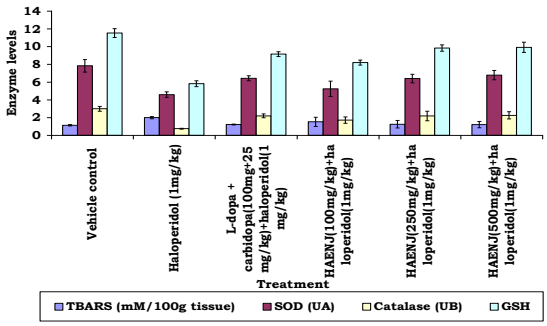

Fig 5: effect of haenj on tbars, sod, cat and gsh levels in haloperidol administered rat brain.

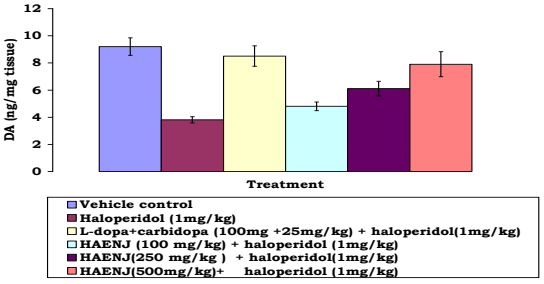

Fig 6: effect of haenj on dopamine levels in haloperidol administered rat

Table 7: effect of haenj on dopamine levels in haloperidol administered rats

Group	Drug treatment	DA (ng/mg tissue)
1	Vehicle control	9.2 ± 0.64
2	Haloperidol (1mg/kg)	3.8a ± 0.23
3	L-dopa+carbidopa (100mg +25mg/kg) + haloperidol(1mg/kg)	8.5*** ± 0.75
4	HAENJ (100 mg/kg) + haloperidol (1mg/kg)	4.8 ± 0.32
5	HAENJ(250 mg/kg) + haloperidol(1mg/kg)	6.1** ± 0.54
6	HAENJ(500 mg/kg)+ haloperidol (1mg/kg)	7.9*** ± 0.92

Values were expressed as mean ± SEM. Statistical significant test for comparison was done by ANOVA, followed Dunnett's't' test. [a] p< 0.001 compared to Group I ,*p<0.05 compared to group II; **p<0.01 compared to group II ; ***p<0.001 compared to group II.

The levels of dopamine in the brains of haloperidol, drug-treated and control groups are shown in Table 7 and Figure 6. The haloperidol-treated rats showed a significant decrease ($P < 0.01$) in dopamine levels in the brain tissue. Oral administration of the extract along with haloperidol administration significantly restored ($P < 0.01$) the dopamine levels to near normal in the brains of the test animals.

For all the parameters studied, *Nardostachys jatamansi* extract administered at a dose of 500 mg/kg body weight showed significant effects. l-dopa and carbidopa also showed a significant effect in all the parameters studied in rats.

DISCUSSION

The antipsychotic induced catalepsy is a sign of the extra pyramidal side effect which is brought about through the inhibition of the nigro-straital dopaminergic neurotransmission.[34] Dopamine and nor adrenaline are involved in the control of motor activity.[35] Haloperidol induce a time dependent catalepsy which was significantly inhibited by hydro alcoholic extract of *Nardostachys jatamansi* , which is indicative of the dopaminergic action of hydro alcoholic extract of *Nardostachys jatamansi. Nardostachys jatamansi* has also been reported to enhance biogenic amines and inhibitory amino acids including a change in the levels of serotonin, 5-hydroxy indole acetic acid, gamma amino butyric acid, and taurine in rat brain. [36] *Nardostachys jatamansi* increase the level of dopamine and its metabolites and increasing the number of dopaminergic D_2 receptors in striatum.[37] Such evidence supports our study and indicates that the extract of *Nardostachys jatamansi* inhibits the symptoms of haloperidol-induced catalepsy in rats.

The induction of free radicals in mammals by haloperidol is well established. It is also well established that the administration of haloperidol leads to an increase in the oxidative stress in the brain tissue.6 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).[38] 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.[39] Free radicals generated in the brain are also reported to influence gene expression, subsequently effecting apoptosis and neuronal death.[38] 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 hydro alcoholic extract of *Nardostachys jatamansi* in haloperidol-induced, cataleptic oxidative stress in rats. 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.[40] 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.[41] 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 *Nardostachys jatamansi* extract increased the activity of these enzymes by quenching the free radicals. Previously *Nardostachys* jatamansi has been reported to be a well known antioxidant and the ethanol extract of *Nardostachys* jatamansi is reported to possess potent antioxidant activity that scavenges free radicals generated after the induction of catalepsy.[42] Significantly lower levels of lipid peroxides in the brains of the drug-treated group and an increased activity of enzymatic and nonenzymatic antioxidants in the brain suggests that the extract reduces oxidative stress. In previous studies *Nardostachys jatamansi* has been reported to have anti lipid peroxidative and protective effect in rat cerebral ischemia. [43,44]

In the present study haloperidol treated animals exhibited an increase in the levels of lipid peroxidation and decreased levels of GSH and protective antioxidant enzymes such as SOD, CAT. Suggesting a possible free radical generation. Hydro alcoholic extract of *Nardostachys jatamansi* revealed the presences of carbohydrates, alkaloids, steroids, sterols, tannins, flavonoids, gums and mucilage, glycosides, terpenes. Terpenes are known to have antioxidant action and they also inhibit mono-amine oxidase enzymes (MAO).[45] Flavonoids also possess MAO inhibitory activity.[46] This activity may me the result of the synergistic effect produced by diffirent constitutent of the extract it is there fore concluded that the tri terpenes of *Nardostachys jatamansi* inhibit haloperidol induced catalepsy and may be investigated further as a putative antiparkinsonian agent. Such evidence supports our study and indicates that the extract of *Nardostachys 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.

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