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NEUROBEHAVIORAL EVALUATION OF NARDOSTACHYS JATAMANSI IN REVERSING METOCLOPRAMIDE-INDUCED CATALEPSY IN RATS.

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ABSTRACT

Oxidative stress has been evidenced to play a vital role in its causation of catalepsy a neurodegenerative disorder. The current study was carried out on Hydroalcoholic rhizomes extracts HAE1 (75:25) & HAE2 (50:50) of *Nardostachys Jatamansi* for its potential in reversing metoclopramide –induced catalepsy in rats. Catalepsy can be induced by metoclopramide (20 mg/kg i.p) in adult Wister rats of either sex. Treatment with hydroalcoholic extract shows a reduction in the cataleptic scores when compared to metoclopramide (toxicant) treated group. There were no signs of toxicity in the animals observed and no significant change in body weight either before or after the treatment. The hydroalcoholic extract HAE1 (500mg/kg) of *N. Jatamansi* shown a maximum reduction in catalepsy than HAE2 (500mg/kg). The biochemical parameters like reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) and glutathione dependent enzymes catalase and superoxide dismutase (SOD) is restored to a normal level in drug treated animals when campare to the animals of toxicant group. Extracts of *N. Jatamansi* upturned the biochemical and behavioral parameters of metoclopramide–induced catalepsy in rats. The contributing factors in reducing catalepsy are due to anti-oxidant & biogenic amines enhancing activity of *N. Jatamansi.*

KEY WORDS: Catalepsy, *Nardostachys Jatamansi,* Anti-oxidant ,Metoclopramide, GSH, SOD.

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INTRODUCTION

Parkinson's disease is one of the major neurodegenerative disorders with symptoms of rigidity, tremor, bradykinesia and stooped posture whose precise cause still unknown. Despite many approaches and efforts to date, no researchers have been successful in developing a cure or at least a modality to check the disease and most of the therapies only provide functional relief.[1] 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.[2] ROS can damage virtually any biological molecule in its surrounding area including; DNA, essential proteins, and lipids of membrane.[3] Parkinson's disease is a neurodegenerative ailment characterized by the loss of dopamine neurons of the *substantia nigra pars compacta*. The phenomenon, which triggers and mediates the loss of nigral dopamine neurons, is not unclear. Neuroleptic-induced catalepsy used as an animal model for screening drugs for Parkinsonism for many years. Catalepsy is defined as the inability of an individual to correct an externally imposed posture. [4] A state characterized by immobility, decreased responsiveness to stimuli. The limbs have a tendency to remain in whatever position they are placed (waxy flexibility). Catalepsy may be linked with psychotic disorders (eg, schizophrenia, catatonia), nervous system due to drug toxicity, and other conditions. The catalepsy test is widely used to calculate motor effects of drugs that act on the extrapyramidal system.[5] Evidence suggests that enormous oxidative stress, free radical formation, genetic susceptibility , and programmed cell death are the major causes for neurodegeneration linked with Parkinson's Disease and other related diseases.[6-7-8] The neuropathology of the illness is based on depigmentation and loss of cell in the dopaminergic nigrostraital area of the brain, with the corresponding decrease in the striatal dopamine (DA) concentration. [9] Besides, dopamine receptor blockade, catecholamine depletion, other neurochemical hypotheses have been proposed for the development of catalepsy such as striatonigral GABAergic (gamma-aminobutyric acid), glutamate, cholinergic and serotonoergic.[10-11] Haloperidol is an antipsychotic drug which is used in the treatment of schizophrenia and other related disorders. It blocks dopaminergic action in the nigrostraital pathway leading to a high frequency of extra pyramidal motor side effects.[12] In animal, metoclopramide induces a behavioral condition known as catalepsy in which the experimental animals are unable to correct externally imposed postures. Metoclopramide-induced catalepsy increase in striatal HVA (striatal homovanillic acid) concentration. The results of the study recommend that metoclopramide causes catalepsy by blocking striatal dopamine receptors. [13] This evidence explains a possible role for antioxidants in the treatment of metacloproamede-induced catalepsy. The organ brain is made up of 70% lipid and any stress is usually manifested by lipid peroxidative damage.[14] The degree of this damage can be used to estimate the degree of cellular harm. Stress-induced lipid peroxidative damage in the brain can be estimated by either determining the amount of peroxidative products or the rates of enzyme-catalyzed reactions neutralizing free radical intermediates such as superoxide dismutase (SOD). In the body SOD is a primary, natural, and free radical scavenging and antioxidant enzyme. The estimation of the activity of such antioxidant enzymes like SOD, catalase, or glutathione peroxidase, can be used to evaluate the therapeutic effects of different antioxidant agents.[15] *Nardostachys jatamansi* (Spikenard) belong to the family *Valirenaceae,* is a flowering plant grows in the Himalayas of India, China and Nepal. The plant grows to 1 meter in height with characteristic pink, bell-shaped flowers. *Nardostachys jatamansi* is found in the altitude of about 3000–5000 meters. In Ayurveda, roots and rhizomes of *N. jatamansi* are used to treat ailments like hysteria, epilepsy, and convulsions.[16] The decoction of the drug is used in neurological disorders, disorders related to cardiovascular system and insomnia.[17] Rhizomes of *Nardostachys jatamansi* are reported to contain a terpenoid ester, Nardostachysin I.[18] The different chemical constituent like sesquiterpenes (Jatamansone, Jatamansic acid), lignans and neolignans are reported to be present in the roots & rhizomes of this plant. [19-20] Much research has been undertaken to evaluate the drug to treat various neurological and cardiovascular disorders in various animal models studies and is used in ayurvedic formulations. It is useful in Alzheimer & cerebral ischemia, have antifungal property, anxiolytic & hypolipidimic activity. It also reported to have antidepressant activity , anticonvulsant activity , antiarrhythmic activity , possess antidiabetic activity and hepatoprotective activity[21-22-23]

MATERIALS AND METHODS

PLANT MATERIAL

Dried roots of *N. jatamansi* were obtained from herbal drug market Hyderabad. The plant were identified and authenticated in march 2011 by experts in the Dept of Botany, Bhavan's new Science College, Narayanaguda, Hyderabad. AP, India.

PREPARATION OF HYDROALCOHOLIC EXTRACT OF N. JATAMANSI

The dried roots of *N. jatamansi* extracted as described by Ahmed et al with ethanol distilled water (75:25) HAE1& (50:50) HAE2 by refluxing in soxhlet for eight hours.[24] The filtrate recieved was concentrated in rotary vacuum evaporator. The extract Screened for its phytoconstituents. The extract obtained was suspended in 1% v/v Tween 80 for oral administration.

PHYTOCHEMICAL STUDY

Table 1
Qualitative chemical tests for phytoconstituents in N. jatamansi Lin

Chemical constituents	Result	Chemical constituents	Result
lignin's	+	volatile oils	+
Alkaloids	+	Steroids	+
Carbohydrates	+	Coumarin	+
Triterpenoids	+	Sapoinins	-
Tannins	+	Resins	-
Protiens	+	Flavonoids	-

EXPERIMENTAL ANIMALS

The adult Wistar rats of either sex, weighing about 150 to 200 gm were purchased from the animal house of the Mahaveer enterprises. The rats were maintained in a room with a 12-hour light/dark cycle in standard cages under controlled temperature (about 26 °C) and humidity (30%–35%). They were fed with the pellet diet. Pure water was supplied to the animals *ad libitium.*

TOXICITY STUDIES

Acute toxicity studies

Random sampling technique was used in selecting rats for acute toxicity study. As per Organization for Economic Co-operation and Development (OECD)-423 guidelines[25] and acute oral toxicity studies was performed with CPCSEA registered no 1330/ac/10/ CPCSEA. The lethal dose (LD)-50 value of the *N. jatamansi* extract was calculated. Six Wistar rats weighing between 155–175 g were used for each dose. The different dose levels of 5 mg, 50 mg, 500 mg, 1000 mg, 2000 mg, and 5000 mg/kg/body weight, per os were selected. Before the dosing, the animals were kept on fasting for 24 hours. After the period of fasting, the fasted body weight of animals was determined as mentioned in paragraph 26 of OECD Guidelines 423 and the dose was calculated according to the reported body weight as per the Annex 2d of OECD Guidelines 423 and as mentioned in Paragraph 23 of OECD Guidelines. The animals were observed for fourteen days for any type of toxic symptoms, such as behavioral changes, convulsions, locomotion and mortality for three days (72 hours).

Repeated oral toxicity studies

In this method repeated oral administration of the substance of interest (extract) over an extended period (daily one dose level during 28 days). To a time covering approximately 10% of the expected life of the animal, drug (*N. jatamansi*) extracts were administered for repeated oral toxicity study. In this repeated oral toxicity studies the dose levels used are lower than acute studies and let the chemicals to mount up in the body before lethality occurs, if the chemical (extract) possesses this ability.[26] Wistar rats weighing between 170–200 g were taken for the study and kept with the temperature in the experimental animal room about 22°C and 12-hour light/dark cycle. Food and water were available in sufficient quantity and were observed each at 72 hour intervals. The rats were divided into two groups, a control group and treated groups, with six animals in each group. The control group received Tween 80 and each treated group received the hydroalcoholic extract of *N. jatamansi* (1000 mg/kg body weight), p.o for 28 days, each animal was weighed every 72 hours. On 28th days, the blood of the rats was collected from the orbital sinus, under ether anesthesia, for biochemical and hematological analysis. The rats were sacrificed by giving anesthesia after collecting blood and organs (brain, liver, pancreas and kidney) were carefully removed and stored in 10% formaldehyde solution before preparing the section. The histopathological studies carried out as per the procedure mentioned by Mukherjee.[27] No death reported during this Toxicity studies.

Table 2
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)			
Hydroalcohalic extract of *N. jatamansi*	5 mg/kg	170	178	No signs of toxicity	No effect	Nil
Hydroalcohalic extract of *N. jatamansi*	50 mg/kg	175	180	No signs of toxicity	No effect	Nil
Hydroalcohalic extract of *N. jatamansi*	500 mg/kg	155	164	No signs of toxicity	No effect	Nil
Hydroalcohalic extract of *N. jatamansi*	1000 mg/kg	165	176	No signs of toxicity	No effect	Nil
Hydroalcohalic extract of *N. jatamansi*	2000 mg/kg	162	170	No signs of toxicity	No effect	Nil
Hydroalcohalic extract of *N. jatamansi*	5000 mg/kg	173	185	No signs of toxicity	No effect	Nil

Observation of acute toxicity study on treatment with hydroalcoholic extract of N.jatamansi in rats for 14 day.

Table 3
Repeated oral toxicity studies

Groups	Hb (gm%)	RBC (millions/mm^2)	WBC (per mm^2)	Differential count			
				Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Control	14.64 ± 0.065	5.31 ±0.164	7489 ±274.10	50.35 ± 1.408	45.19 ± 0.835	2.337 ± 0.6150	2.169 ± 0.5428 ns
Test	14.65 ± 0.082 ns	5.075 ± 0.144 ns	7712 ± 246.10ns	52.19 ± 1.169ns	41.85 ± 1.449 ns	2.671± 0.6148 ns	3.502± 0.6709 ns

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.

NEUROBEHAVIORAL STUDIES
Experimental design
Male Wistar adult rats weighing between 150–200 g selected for this study were divided randomly into five groups each group containing six animals. Group I (control Group) and received the vehicle, 1% Tween 80 solution. Group II (Cataleptic control Group) and received metoclopramide alone with no drug treatment. Group III (positive control Group) and received a combination of l-dopa and carbidopa (100 mg + 25 mg/kg by intraperitoneal administration). Groups IV received extract HAE1 (500 mg/kg) and Group V, received extract HAE2 (500 mg/kg) of *N. jatamansi.* The Catalepsy was induced by the intraperitonial administration of metoclopramide 20 mg/kg body weight, 30 minutes after the administration of standard drug and test drug (*NJ*, Tween 80 solution and l-dopa, carbidopa). The above experimental procedure was repeated for 15 days. Studies on the behavior of the rats carried out at room temperature without any external disturbances. The severity of catalepsy was assessed every 30 min, thereafter for a total duration for 180 min for metal bar test and 90 minutes for block method. Catalepsy of each animal was measured in a stepwise manner using a scoring method described below. After completion of the studies, rats were sacrificed by cervical dislocation and the entire brain was dissected out and blood removed by cleaning with ice-cold saline. The brain tissue's (10%) homogenate was prepared by using 0.025 M Tris–HCl buffer at a pH 7.5 and used to assess the activities of thiobarbituric acid reactive substances (TBARS). The brains tissue's (10%) homogenate prepared in 0.2 M phosphate buffer at pH 8.0 used to assess enzyme activity studies.

Measurement of catalepsy by block method.[28]
Three step scoring methods followed for this study, which is as fallows.
Step I
The rat was removed from the home cage and placed on a table, when pushed or touched gently on the back of the rat, if the rat failed to move from the place a score of 0.5 was assigned.
Step II
In the step two, if the rat failed to correct the posture within the 15 seconds when the front paws of the rats were placed alternately on a 3 cm high block, a score of 0.5 for each paw was added to the above score of step I.
Step III
In the step three, if the rat failed to correct the posture within the 15 seconds when the front paws of the rat were placed alternately on a 9-cm high block, a score of 1 for each paw was added to the above scores of steps I and II. Thus, the maximum score for an animal was 3.5 (cut off score) and that reflects maximum catalepsy in an animal (Table 4).

Assessment of catalepsy by metal bar method
In Metoclopramide-induced cataleptic rats behavioral evaluation was studied according to the manner described by Kulkarni.[29] Measurement of Cataleptic behavior was done with the help of a high bar test method. Catalepsy score was measured for 180 min at 60 min intervals after Metoclopramide administration, by softly, placing both fore paws of the cataleptic rat over a metal bar (diameter 4 mm suspended 6 cm above the table top). The intensity of the catalepsy measured by counting the time (in seconds) until the rat brings both forepaws down to the tabletop, with a maximum cutoff time of 3 minutes. Finally, scores at different time points (0, 60,120 and 180 minutes after Metoclopramide injection) were added and expressed as a cumulative catalepsy score for comparison purposes (Table 5).

ANTIOXIDANT STUDIES
➢ ***Evaluation of Lipid Peroxidation Products***
Colorimetrically, Lipid peroxidation was estimated by counting TBARS in brain tissue as described by the Niehaus and Samuelson.[30] In brief, in order to calculate TBARS the supernatant of the tissue homogenate was mixed with a reagent know as tertiary butanol–trichloroacetic acid–hydrochloric acid (TBA–TCA–HCl) .The above mixture kept in boiling water bath for a period of 15 minutes. After bringing the temp down to room temp, the tubes were centrifuged for 10-15 minutes and the supernatant taken for measurement of colour. The color developed due to reaction between TBA–TCA–HCl and supernatant of the tissue homogenate was examine at 535 nm using a UV spectrophotometer against a reagent blank and expressed as mM / 100g tissue.

Catalase Assay
Catalase (CAT) was assayed Colorimetrically at a wave length 620 nm and was expressed as micromoles of hydrogen peroxide (H_2O_2) consumed per minute per mg of protein, as per the method of Sinha.[31] The total volume 1.5 ml of this reaction mixture contained 0.1 ml of tissue homogenate, 0.4 ml of 2 M hydrogen

peroxide (H_2O_2) and 1.0 ml of 0.01 M pH 7 phosphate buffer. The reaction comes to end by the addition of 2 ml of dichromate-acetic acid reagent (a mixture of glacial acetic acid and 5% potassium dichromate mixed in the ratio of 3:1).

➢ ***Assay of SOD***

This assay for SOD is a reduction reaction, based on inhibition of the reduction of nitroblue tetrazolium to blue formazan by superoxide anions which is mediate by SOD, explained by Beauchamp and Fridovich.[32] The total amount of protein available in the homogenate was quantified according to the procedure explained by Lowry.[33] The SOD was expressed in milligrams of TP (total protein).

➢ ***Assay of GSH***

Procedure explained by Ellman is followed to determine reduced glutathione (GSH).[34] 1 ml of supernatant was mixed with 3 ml of phosphate buffer (0.2 M at pH 8.0) and0.5 ml of Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB). The absorbance of visible light measured at 412 nm. Units of GSH activity determined were expressed as nM GSH formed/g tissue.

STATISTICAL ANALYSIS

Taking six animals in a group the animals divided into five groups. The data obtained from the study were evaluated by ANOVA (analysis of variance) followed by Dunnett *t*-test. All the values are expressed as mean ± standard error of mean (SEM). $P < 0.05$ was taken to be the level of significance; $P < 0.001$ was taken to be the level of highly significance.

RESULTS

Table 4

Result of Hydroalcoholic extract of N. jatamansi on Metoclopramide induced catalepsy by block method

Drug treatment	15 min	30 min	60 min	90 min
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Metoclopramide 20 mg/kg	1.61 ± 0.12	2.50 ± 0.22a*	3.63 ± 0.24a**	3.69 ± 0.68a**
L-dopa and Carbidopa + Metoclopramide 20mg/kg	0.56 ± 0.18b*	1.13 ± 0.15b**	0.98 ± 0.46bb**	0.67 ± 0.32b**
N. jatamansi HAE 1(500 mg) + Metoclopramide 20mg/kg	0.92 ± 0.24b*	1.55 ± 0.19b**	1.10 ± 0.33b**	0.99 ± 0.34b**
N. jatamansi HAE 2 (500 mg) + Metoclopramide 20mg/kg	1.45 ± 0.39	2.19 ± 0.29b*	1.75± 0.23b*	1.52 ± 0.42b**

Values are mean ± SEM (n = 6). Statistical analysis by one-way ANOVA, followed by Dunnett's multiple comparison tests.
P < 0.05,**P < 0.01, a=compare with Group I,b compare with Group II

Abbreviations

SEM, standard error of mean; Min, minutes.

Table 5

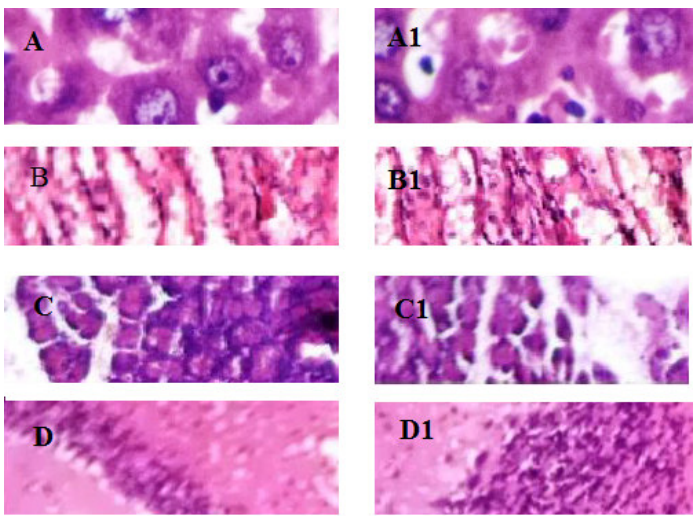
Result of Hydroalcoholic extract of N. jatamansi on Metoclopramide induced catalepsy by metal bar test

Drug treatment	0 min	60 min	120 min	180 min
Control	8.52 ± 1.090	7.18 ± 1.141	5.18 ± 0.795	5.68 ± 0.559
Metoclopramide 20 mg/kg	4.85 ± 0.874	120.4 ± 3.37a**	145.2 ± 3.56a**	166 ± 5.59a**
L-dopa and carbidopa + Metoclopramide 20mg/kg	6.51 ± 0.79	31.51 ± 3.54b**	26.53 ± 2.142bb**	23.02 ± 1.35b**
N. jatamansi HAE 1 (500 mg) + Metoclopramide 20mg/kg	5.91 ± 0.424	43.44 ± 3.14b**	36.25 ± 4.125b**	28.32 ± 2.149b**
N. jatamansi HAE 2 (500 mg) + Metoclopramide 20mg/kg	8.18 ± 0.655	65.02 ± 4.31b**	58.85 ± 3.49b**	48.02 ± 3.10b**

Values are mean ± SEM (n = 6). Statistical analysis by one-way ANOVA, followed by Dunnett's multiple comparision tests.
P < 0.05,**P < 0.01 , a=compare with Group I,b compare with Group II

Abbreviations

SEM, standard error of mean; Min, minutes.



Histopathological slides of organs. Figure A-A1, B-B1 , C-C1 and D- D1 have not shown any differences in the architecture of liver, kidney, pancreas and Brain respectively on repeated oral toxicity evaluation of *N jatamansi* treatment for 28 days in wistar rat.

Table 6
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	1.25 ±0.10	7.25 ±0.90	3.95±0.28	12.01 ±0.49
Metoclopramide 20 mg/kg	2.01 ±0.14***	4.60 ±0.33***	0.69±0.08***	4.79 ±0.33***
l-dopa and carbidopa +Metoclopramide 20 mg/kg	1.24 ±0.07***	6.98 ±0.30***	2.80 ±0.31***	10.17±0.29***
N. jatamansi (HAE1 500mg) +Metoclopramide	1.26±0.08***	6.83 ±0.52***	2.71 ±0.29**	9.72 ±0.49***
N. jatamansi (HAE1 500mg) mg) +Metoclopramide	1.30 ±0.23**	6.7 ±0.40**	2.51±0.27**	8.52±0.39**

Values are 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_2O_2 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.

The phytochemical studies of hydroalcoholic root extract of *N. Jatamansi* revealed the presence of phytochemical constituents like carbohydrates, Alkaloids, Triterpenoids, Tannins, Protiens, lignin's, Steroids, Coumarin, volatile oils. Acute oral toxicity carried out according to OECD guidelines 423 (acute toxicity class method). There were no signs of toxicity in the animals observed and no significant change in body weight either before or after experiment (table 2). The LD50 test of *N. Jatamansi* extract was found to be a dose greater than 5000 mg/kg body weight on oral administration. On the other study in repeated oral toxicity a dose of 1000 mg/kg body weight was given for 28 days p.o. The parameters like Hematological (WBC,hemoglobin, RBC, monocytes, neutrophils, lymphocytes and eosinophils) of *N. Jatamansi* extract treated animals were almost alike when compared with the animals of normal control group (Table 3). Histopathological examination of the organs , liver, kidney, pancreas and brain did not shown any difference in their normal structure, explaining about the safety of the drug (Figures A-A1, B-B1 , C-C1 and D- D1) on oral administration of the drug to normal animals. On the basis of these results a dose of 500 mg/kg bodyweight of HAE1, HAE2 were selected for this evaluation. The outcomes of this study in terms of cataleptic scores are specified in Table 4 and 5, assessed by two different method, block method and metal bar test, respectively. Metoclopramide (toxicant) induced catalepsy significantly (P < 0.01) at a dose of 20 mg/kg (intraperitoneal administration). Significant reversal to normal parameter in metoclopramide-induced catalepsy was observed with the administration of *N. jatamansi* hydroalcoholic extracts and standard drug in combination of l-dopa and carbidopa. The maximal reversal (P < 0.01) in catalepsy was observed in the group receiving HAE1of *N. Jatamansi* at a dose of 500 mg/kg. However, there was reduction in the cataleptic scores of HAE2 at a dose of 500 mg/kg, less compare to the results observed with HAE1 500 mg/kg. The quantity of lipid peroxidation products and antioxidants in the brains of metoclopramide treated, drug-treated and control groups are shown in (Table 6). The metoclopramide-treated animals showed a significant increase (P < 0.01) in TBARS and there was also a reduction (P < 0.01) in SOD, CAT, and GSH in the brain tissue. Oral administration of the extract along with metoclopramide 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 HAE1& HAE2 administered at doses of 500 mg/kg bodyweight, HAE1 showed significant effects along with standard L-dopa and Carbidopa which also showed a significant effect in all the parameters studied in rats. Where as HAE2 was less significant when compared with the HAE1.

DISCUSSION

Among the body systems which are susceptible to free radical damage, the most important one is the CNS system because of high oxygen consumption of the brain, because it is rich lipid content, and the relative lack of antioxidant enzymes as compared with other tissues.[35] Facts also indicate that ROS may stimulate extracellular release of excitatory amino acids.[36] Glutamate is the most important excitatory amino acid in the brain which works through various types of ionotropic receptors, the significant being *N*-methyl *D*-aspartate (NMDA) receptors. There seems to be a two directional relationship between the ROS production and the release of excitatory aminoacids.[37] Free radicals generated in the brain are also reported to play a role in gene expression, consequently effecting apoptosis and neuronal death.[38] In the brain, an arrangement of cellular defense systems exists to counterbalance the ROS. These comprises of enzymatic and non-enzymatic antioxidants that lower the concentration of free radical species and repair oxidative cellular damage. The brain is well-known to synthesize molecules like glutathione and NADPH. Glutathione functions as a main antioxidant in tissue defense against free radicals responsible for the brain damage . However, the concentration of glutathione is, relatively, less in the brain compared to the other organs.[35] The natural antioxidant system present in brain can be in the form of enzymes like peroxidase, catalase, superoxide dismutase or low molecular weight antioxidants , ascorbic and lipoic acids, carotenoids or chelating agents.[38] Free radical scavengers or antioxidants function as biological protector for essential molecules by two mechanisms either neutralizing reactive species

before they mutilate a molecule and/or they repair damage that has been occured. This study demonstrates the antioxidant effects of an hydroalcoholic root extracts of *N. Jatamansi* in Metoclopramide-induced, cataleptic oxidative stress in rats. Several neuroleptics including metoclopramide and haloperidol are able to exert antioxidant and/or pro-oxidant action in vivo.[39] Metoclopramide is a neuroleptic/antiemetic drug with antagonistic effects on both dopamine and 5-HT receptors.[40,-41,-42,-43] Administration of metoclopramide has been reported to cause tardive dyskinesia and extrapyramidal movement disorders in mammals.[44- 45] Common adverse effects associated with metoclopramide therapy include extrapyramidal effects such as oculogyric crisis.[46] It has been reported that the cataleptic effects of metoclopramide are apparently mediated by dopamine receptors restricted postsynaptically on strial neurons. The increase in SOD observed in this study supports this concept. Superoxide formation is a main factor in toxicity caused by oxygen and the superoxide dismutase enzyme constitutes an important defense against it. Decreased activity of antioxidant enzymes, like SOD, glutathione peroxidase and catalase, in the brain leads to the increase of oxidative free radicals resulting in degenerative effects.[47] On the other hand an increase in these enzymes under normal conditions would correspond to increased antioxidant activity and a protective mechanism in neuronal tissue, thus, constitute the first line of protection against oxidative stress in the body. In the presence of a free radical-quenching agent, the generation of the antioxidant enzymes is minimized. So, any decrease in cataleptic scores and SOD activity in the drug treated groups indicates the ability of the drug extract (*N. Jatamansi*) to fight oxidative stress in tissues of brain and reduce the severity of metoclopramide-induced catalepsy. The changed balance of the antioxidant enzymes caused by the decrease in CAT, SOD, GSH functions may be responsible for the inadequacy of the antioxidant defenses in fighting ROS mediated damage. The decreased activities of CAT and SOD may be a reason to increased production of hydrogen peroxide and oxygen by the autooxidation.[48] It has been suggested that these enzymes play an important role in maintaining physiological levels of O_2 and H_2O_2 by speeding up the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides. Treatment with *N. jatamansi* extracts increased the activity of above enzymes by quenching the free radicals. Formerly *N. jatamansi* has been reported to possess antioxidants and the ethanol extract of *N. jatamansi* is reported to possess potent antioxidant activity and scavenges free radicals generated after the induction of the catalepsy.[49] Lower levels of lipid peroxides in the brains of the drug-treated group and increased activities of enzymatic and nonenzymatic antioxidants in the brain recommend that the extract reduces oxidative stress. In prior studies *N. jatamansi* has been reported to have antilipid peroxidative and protective effect in rat cerebral ischemia' it also reported that it enhance biogenic amine activity' decrease the level of dopamine and its metabolites and also increasing the number of dopaminergic D2 receptors in striatum.[50-51-52-53] Such facts support this study and indicate that the hydroalcoholic extracts of *N. jatamansi* attenuate the symptoms which are associated with metoclopramide-induced catalepsy in rats. The action, by which the amelioration takes place, may be due to one or more pharmacological/biochemical mechanisms which are taking place. In conclusion, the brain exhibits various types of morphological and functional alterations during oxidative stress, a single factor implicated in the pathogenesis of many CNS disorders. Treatment of such type of neuronal disorders with *N. jatamansi* plant extracts decreases lipid peroxidation and increases the antioxidants in the brain.

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CONCLUSION

The conclusion of this study suggest the possible antioxidant role of hydroalcoholic extracts of *N. jatamansi* in managing behavioral and neurochemical changes during oxidative stress. Since the catalepsy test has predictive value regarding extrapyramidal effects cataleptic rats, the possibility of pharmacological interactions between metoclopramide and *N. jatamansi* extract should be investigate further in clinical studies to use this drug as a possible cure to catalepsy.

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CONFLICT OF INTEREST

None

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