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Research Article

EFFECT OF HYDROALCOHOLIC EXTRACT OF *ACHYRANTHES ASPERA* ON HALOPERIDOL-INDUCED PARKINSON'S DISEASE IN WISTAR RATS

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ABSTRACT

Objective: Prolonged usage of neuroleptics in psychotic disorders such as schizophrenia provokes extrapyramidal symptoms that are also seen in Parkinson's disease. An attempt has been made to study the neuroprotective role of *Achyranthes aspera* hydroalcoholic (HA) extract on haloperidol-induced Parkinson's symptoms in Wistar rats.

Methods: The present study deals with the antiparkinson effect of HA extract of *A. aspera* on haloperidol (2 mg/kg intraperitoneal administration)-induced catatonia in Wistar rats. The motor coordination in case of haloperidol-treated animals was studied by performing rotarod test and hang test. Dopamine and 3,4-dihydroxyphenylacetic acid were estimated using an electrochemical detector and high-performance liquid chromatography. The antioxidant status was also assessed to know the neurotoxicity of haloperidol by estimating the levels of lipid peroxidation, superoxide dismutase, glutathione (GSH) peroxidase, and reduced GSH by performing individual assays.

Results: All these assessments were done on 24 Wistar rats which were divided into four groups (n=6). HA was administered at 200 and 400 mg/kg doses, 30 minutes before haloperidol treatment for 20 days. HA significantly (*p<0.05, **p<0.01) improved the antioxidant status.

Conclusion: The results shown that HA shows a protective role in haloperidol catalepsy and also possess antioxidant property.

Keywords: *Achyranthes aspera*, Haloperidol, Catatonia behavior, Parkinson's disease, Levodopa.

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INTRODUCTION

Parkinson's disease (PD) is primarily characterized by degradation of dopamine-carrying neurons in the substantia nigra with the extrapyramidal symptoms such as tremors, bradykinesia, rigidness, and inability to maintain the normal posture [1]. The neuronal death in PD is due to the damage to free radicals, Lewy's bodies formation [2]. In general, a combination therapy of synthetic drugs is more effective in Parkinson's treatment. Levodopa is the first-line drug of PD and still long-term use of levodopa has many side effects [3]. Prolonged usage of the antipsychotic drugs such as haloperidol causes dopamine receptor blockade in the corpus striatum, thus producing the extrapyramidal effects similar to that in PD. Catalepsy produced by neuroleptic agents such as haloperidol is a widely accepted animal model for screening drugs for parkinsonism [4]. Catalepsy is the loss or decrease of the ability of an animal to regain its externally imposed posture. Gamma-aminobutyric acid deficiency and cholinergic dysfunction are proved to be cause for catalepsy [5].

Achyranthes aspera possesses diuretic, laxative, antiasthmatic, hepatoprotective, nephroprotective, bronchoprotective, and antiallergic properties [6]. The methanolic extract of this whole plant has been confirmed for its anti-inflammatory, antioxidant, and antidepressant activities [7,8] previously and now its hydroalcoholic (HA) used to find its role in enhancing the dopaminergic transmission and antioxidant capacity in PD.

METHODS

Preparation of plant extract

The whole plant of *A. aspera* was obtained from Tirunelveli district in Tamil Nadu, and authentication (voucher specimen number - PARC/2015/3194) was done by Prof. Jayaraman P, Ph.D, Plant Anatomy Research Centre, Tambaram, Chennai-45. The plants were cleaned, air dried, and powdered to fine in an automix blender and are deep frozen. The *A. aspera* powder is extracted using HA in a Soxhlet extractor and evaporated to a concentrate by rotary evaporator at 40°C and preserved in a cool, dry place until its use [9].

Animals

Twenty four Wistar albino rats of 200-250 g weight were procured from King's Institute, Guindy. In accordance to the IAEC guidelines, the rats were placed in standard laboratory conditions with room temperature of 25±1°C and 60% humidity. A standard balanced diet with water is provided to the test animals. The project proposal was approved by Institutional Animal Ethical Committee (IAEC 163/2015).

Drug treatment and experimental design

Twenty four animals were grouped into four, having six in each group (n=6). Group I received haloperidol (1.0 mg/kg, i.p), Group II received haloperidol (1.0 mg/kg, i.p) + L-DOPA + carbidopa (100+25 mg/kg, p.o) suspended in 1% tween 20, and Groups III and IV received 200 and 400 mg/kg HA (p.o), respectively, 30 minutes before haloperidol injection for 20 days.

After the treatment for 20 days, all four groups of animals underwent the behavioral assessment tests. Then, cervical decapitation was performed to isolate the striatum of the brains of the animals, and homogenate was prepared using ice-cold phosphate-buffered saline solution and stored.

Catalepsy behavioral study

Haloperidol (1.0 mg/kg i.p.) used in the catalepsy induction and the rats were assessed for every 30 minutes up to 120 minutes by performing a standard bar test. Haloperidol among the antipsychotics produces a moderate cataleptic effect. Catalepsy was given in scores for

the imposed position of the front limbs placed on a 3 and 9 cm high wooden bar of 1 cm wide. When the both front paws were taken from the bar or if the animal moves its head, this can be termed as the end point. Levodopa (100 mg/kg) was taken as the standard drug, and the *A. aspera* HA extract was administered to three different groups (n=6 in each group) at a dose of 200 and 400 mg/kg p.o., respectively, 1 hr before to the dosing of haloperidol.

If the animal is unable to regain its posture for about 10 seconds, it was considered to be cataleptic. Stage I – Rat is able to move freely on the table it is placed, score = 0. Stage II – Rat moves when touched or pushed, score = 0.5. Stage III – If the rat is unable to correct its posture in 10 s when its front paws are set alternately on a 3 cm high block, the score = 0.5 for each paw with a total 1 score. Stage IV – Rat could not remove its front paws when placed on a 9 cm high block, score = 1 with a total score = 2 for this stage [10].

Rotarod test

The rotarod apparatus is a rotating rod of 70 cm length and 3 cm diameter at the height of 50 cm above the floor and has four divisions where four animals can be placed each time during the study. Before the assessment, all the rats underwent five trials by maintaining rate of rotation at 30 rpm. The control rat remains on the rod for about 5 minutes. The treated rats were kept on the rotating rod at regular intervals, and the time of the fall-off time was noted. The cutoff time for the test was 5 minutes [11].

Hang test

The neuromuscular strength of the animals was determined by the hang test. The rat was lifted by its tail and left on a horizontal grid mounted 20 cm above the hard surface and given support until it grabs on the grid with its fore and hind paws. The grid was then inverted for the rat to hang upside down [12]. The rats are to be stayed on the grid for about 30 seconds to 1 minute. The maximum hanging time was recorded as mentioned below:

$$\text{Percentage of hanging time} = \frac{\text{Maximum hanging time}}{30 \text{ seconds}} \times 100$$

Assessment of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) levels

The dissected brain samples were weighed, frozen at 80°C until assay, and homogenized in one milliliter ice-cooled 0.1 mmol/L perchloric acid solution containing 0.2 µg/ml L-isoproterenol hydrogen and 0.1 mmol/L ethylenediaminetetraacetic acid (EDTA). Tissue homogenates were centrifuged at 15,000 ×*g* at 4°C for 30 minutes, and the supernatant was filtered and stored at −80°C until assay. High-performance liquid chromatography with an electrochemical detector and 25 cm × 0.5 cm I.D column used in the assessment of dopamine and DOPAC levels. The sample peak obtained is compared with the standard peak and expressed in microgram per gram of tissue weight [13].

Estimation of lipid peroxidation (LPO) in brain homogenate

Estimation of malondialdehyde

Three hundred microns of 10% trichloroacetic acid (TCA) were added to 150 µL of each sample and centrifuged at 1000 rpm for 10 minutes at 4°C. About 300 µL of the supernatant was incubated with 300 µL 0.67% thiobarbituric acid at 100°C for 25 minutes. The mixture was cooled for 5 minutes and the thiobarbituric acid reactive substances (TBARS) concentration as pink stains was read in a spectrophotometer at 535 nm [14].

Assay of superoxide dismutase (SOD)

SOD was assessed by the inhibition of formation of nicotinamide adenine dinucleotide (NADH) - phenazine methosulphate nitroblue tetrazolium formazon complex. NADH was assessed after 90 s of incubation and the reaction was terminated by the addition of glacial acetic acid. The color formed as the end point of the reaction was extracted into the butanol layer and measured at 520 nm [15].

Assay of catalase (CAT)

The tissue was homogenized in isotonic buffer (pH - 7.4) and centrifuged at 1000 ×*g* for 10 minutes. 20 µl of 100-fold diluted tissue supernatant added to 980 µl of the assay mixture; the assay mixture consists of 900 µl of 10 mmol/L of H_2O_2, 50 µl of Tris-HCl buffer (pH - 8), and 30 µl of water. The degree of decomposition of H_2O_2 was monitored spectrophotometrically at 240 nm [16].

Estimation of glutathione peroxidase (GPx)

A volume of 0.1 ml of the diluted tissue was incubated at 37°C with reaction mixture consisting of 0.2 ml of each EDTA, sodium azide, and H_2O_2. 0.5 ml of TCA was added to this mixture to stop the reaction and then centrifuged at 2000 rpm. 4 ml of disodium hydrogen phosphate and 0.5 ml 5,5'-dithiobis nitro benzoic acid (DTNB) were added to 0.5 ml of supernatant, and the color formation was recorded at 420 nm in a spectrophotometer [17].

Activity was expressed as µ moles of glutathione oxidized/minutes/mg protein.

Assay of reduced glutathione (GSH)

To 2 ml of the tissue homogenate and KCl mixture, add 4 ml of cold distilled water and 1 ml of 50% TCA. The contents were centrifuged at 3000 rpm for 15 minutes, and from this 2 ml of the supernatant was collected and 4 ml of 0.4 M Tris buffer (pH 8.9) and 0.1 ml of 0.01 M DTNB were added, the absorbance was read 412 nm against the blank reagent. For blank readings, 2 ml of distilled water was used in the place of tissue homogenate.

Total GSH was calculated using the formula:

$Co=(A*D)/E$

Where, A is absorbance at 412 nm, D is dilution factor, and E is the molar extinction coefficient (C=13,000 M^{-1} CM^{-1}); Co is the concentration of GSH [17].

Statistical analysis

One-way analysis of variance followed by Dunnett's test was employed for the analysis of data in catalepsy test, biochemical and other behavioral parameters. *p<0.01 and **p<0.05 were considered statistically significant.

RESULTS

An increase in the degree of catalepsy was noticed in the haloperidol group after 60 and 90 minutes of administration. The score was significantly reduced after 60 minutes with the test drug HA at 200 and 400 mg/kg doses (Fig. 1). During the period of observation till 120 minutes, there has been a significant reduction. The 400 mg/kg treated group exhibited maximum reduction in the catalepsy.

Fig. 2 shows the results of rotarod test. The retention time was reduced in the haloperidol-tested animals and was significantly improved in HA-treated groups. Among these two groups, maximum increase in the retention time was noted in 400 mg/kg group.

Fig. 3 shows the results of hang test. The fall-off time was reduced in the haloperidol group and it was improved significantly by treatment with the extract.

Table 1 shows the dopamine, DOPAC, and homovanillic acid levels which were decreased in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyri dine-treated group and were significantly (*p<0.05, 0.01) increased on hydroalcoholic extract of *A. aspera* (HA) treatment, especially at 200 mg/kg dose.

Striatal TBARS and antioxidant status are presented in Table 2. The striatal TBARS was significantly increased in haloperidol-treated

Table 1: Effect of hydroalcoholic extract of *Achyranthes aspera* (HA) on Dopamine and DOPAC levels

Groups (n=6)	Dose (mg/kg)	Dopamine (mg/g of brain tissue)	DOPAC
I	Haloperidol (1 mg/kg)	1.56±0.04	0.73±0.02
II	Haloperidol+L-DOPA+carbidopa 1+100+25	3.72±0.01**[a]	1.26±0.15**[a]
III	HA (200 mg/kg)+haloperidol 200+01	4.38±0.14*[b]	1.68±0.04*[b]
IV	HA (400 mg/kg)+haloperidol 400+01	6.90±0.18**[b]	1.94±0.01*[b]

[a]Compared with Group-I, [b]Compared with Group-II, *p<0.05, **p<0.01. DOPAC: 3,4-dihydroxyphenylacetic acid, HA: Hydroalcoholic

Table 2: Effect of hydroalcoholic extract of *Achyranthes aspera* (HA) on Anti-oxidant enzymes

Groups (n=6)	Dose (mg/kg)	SOD (µ/mg protein)	LPO (n moles/mg protein)	CAT (µ/mg protein)	GPx (µ/mg protein)	GSH (µ/mg protein)
I	Haloperidol (1 mg/kg)	0.93±0.84	2.56±0.04	0.12±0.01	3.28±0.13	0.09±0.04
II	Haloperidol+L-DOPA+carbidopa1+100+25	1.96±0.12**[a]	4.39±0.07**[a]	0.16±0.04**[a]	7.79±0.09**[a]	0.22±0.06**[a]
III	HA (200 mg/kg)+haloperidol 200+01	2.38±0.31*[b]	3.86±0.01*[b]	0.20±0.04*[b]	9.72±0.12*[b]	0.39+0.01*[a]
IV	HA (400 mg/kg)+haloperidol 400+01	3.88±0.26*[b]	2.92±0.04**[b]	0.37±0.02**[b]	10.13+0.23**[b]	0.60+0.04**[b]

[a]Compared with Group-I, [b]Compared with Group-II, *p<0.05, **p<0.01. HA: Hydroalcoholic, SOD: Superoxide dismutase, LPO: Lipid peroxidation, CAT: Catalase, GPx: Glutathione peroxidase, GSH: Glutathione

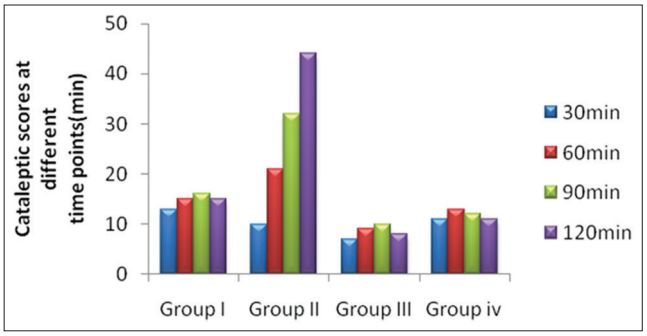

Fig. 1: Effect of hydroalcoholic extract of *Achyranthes aspera* (HA) on Catalepsy behavior in mice

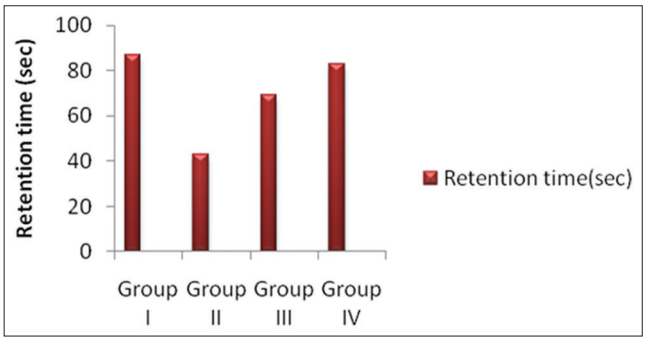

Fig. 2: Effect of hydroalcoholic extract of *Achyranthes aspera* (HA) on Rota rod test

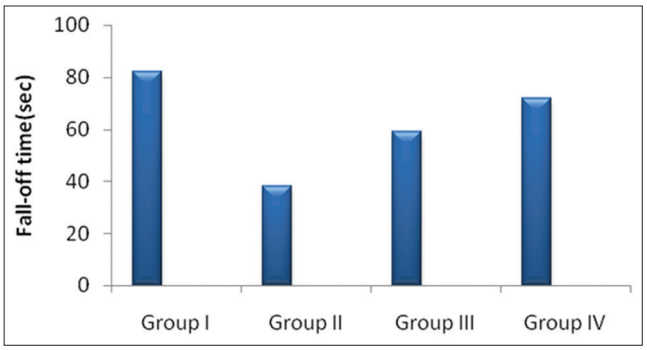

Fig. 3: Effect of hydroalcoholic extract of *Achyranthes aspera* (HA) on Hang test

group of mice when compared to control group. HA reduced the TBARS level. SOD, CAT, GPx, and GSH activities were significantly decreased in haloperidol-treated group when compared to control group and were again restored significantly on treatment with HA at 200 and 400 mg/kg doses.

DISCUSSION

Free radical damage to the central nervous system (CNS) is due to its high oxygen utility, increased lipid content and inadequate antioxidant enzymes than compared to other tissue. The free radical generation in the brain influences gene expression, subsequently leading to apoptosis and neuronal death. In the brain, an array of cellular defense systems, i.e., enzymatic and non-enzymatic antioxidants exists to counterbalance the generation of reactive oxygen species [2].

Studies show that prolonged treatment with antiparkinson drugs such as dopamine agonist, dopamine replenishment therapy, and monoamine oxidase inhibitors leads to severe side effects and decrease in the sensitivity for the therapy [18]. Further, typical neuroleptic drugs such as chlorpromazine, haloperidol, and reserpine use in schizophrenia lead to decrease in dopamine content and state of catalepsy. The cataleptic induction model by neuroleptics in rodents is the widely accepted model to test the extrapyramidal side effects of antipsychotic agents. Evidences indicate that drugs which produce or reduce the catalepsy in rodents might also show the same effects in human beings. The conversion of levodopa to dopamine in serotonin neurons was proved as a compensatory measure in PD [19,20].

Previous studies have shown that dopamine receptors in the striatum are involved in the neuroleptic-induced catalepsy [5]. It has been demonstrated that the cataleptic effects of haloperidol are mediated by dopamine receptors of the striatal neurons. It was also reported that the administration of haloperidol provokes an oxidative stress in the brain tissue. An increase in SOD concentration in the present work supports the concept. Under normal conditions, decrease in the activities of SOD, GPx, and CAT enzymes in the brain leads to the accumulation of oxidative free radicals resulting in degenerative effects [6]. At normal conditions, increased concentration of these enzymes would represent the rise in antioxidant activity and exhibits a protective mechanism in neuronal tissue, thus constituting the first line of defense against oxidative stress in our body. Hence, any decrease in the degree of catalepsy and rise in the SOD activity in the drug-treated groups indicates the ability of the drug extract to protect against the oxidative stress in the brain tissue and reduce the severity of haloperidol-induced catalepsy. Treatment with *A. aspera* extract increased the activity of these enzymes by quenching the free radicals.

Researches have shown that *A. aspera* possesses potent antioxidant activity that scavenges free radicals generated after the induction of catalepsy. Lower levels of LPO in the brains of the drug-treated groups and increased activities of enzymatic and non-enzymatic antioxidants suggest that the extract reduces oxidative stress. The methanolic extract of *A. aspera* showed no signs of lethality up to 2000 mg/kg. Hence, initially, two doses, i.e., 200 and 400 mg/kg of hydroalcoholic extract of *A. aspera* were selected to study their effect on haloperidol-induced parkinsonism in Wistar albino model. At a dose of 400 mg/kg, the HA extract showed a significant reduction (*p≤0.01) of the parkinsonism effect in Wistar albino rats.

CONCLUSION

During oxidative stress, numerous morphological and functional alterations are observed in the pathogenesis of many CNS disorders. Treatment of such neuronal disorders with *A. aspera* whole plant extract significantly decreased LPO and increases the antioxidants in the brain significantly. The findings of this study suggest the possible antioxidant role of *A. aspera* extract in overcoming the neurochemical and behavioral changes during oxidative stress. Since the catalepsy test has predictive value regarding extrapyramidal effects, the possibility of pharmacological interactions between haloperidol and *A. aspera* whole plant extract because of its effectiveness in PD can be further extrapolated to clinical studies for better therapy in humans.

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