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Neuroprotective Effect of Acorus Calamus on a Rat Model of Parkinson's Disease Induced by 6-Hydroxydopamine

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ABSTRACT

The well known pathology of PD is the preferential death of dopaminergic neurons in substantia nigra pars compact (SNpc), causing a decrease in striatal dopamine (DA) and presence of intracellular a – synuclein- positive inclusions called Lewy bodies[1]. *Acorus calamus* is commonly known as "bach" or"ugragandha" in India. Roots, rhizomes and leaves have been used in the Indian systems of traditional medicine for hundreds of years. The rhizome contains active ingredients possessing Insecticidal, Neuroprotective, Antifungal, Antibacterial and Allelopathic properties. Acorus calamus is a traditional medicinal plant that is commonly used for treating central nervous system abnormalities. Methanolic extract of rhizomes of Acorus calamus was administered at three level dose (250, 500 & 750 mg/kg) on a rat model of Parkinson's disease induced by 6-hyroxydopamine. The results showed that Acorus calamus markedly improved the locomotor, motor balance, coordination in 6-OHDA-lesioned rats. The expression of tyrosine hydroxylase (TH) in substantia nigra (SN) and the content of extracellular dopamine (DA) in striatum were also significantly increased after AC treatment. Moreover, significant AC increased the level of DJ-1 & decreased the α-synuclein levels in western blotting analysis. We propose that AC has potent anti-Parkinson property possibly through increasing TH, DA, DJ-1& Decreasing α-synuclein in PD rats[2].

Keywords: Parkinson's Disease(PD), Acorus Calamus (AC), Dopamine (DA)

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Introduction

Neurodegenerative disorders remain an important source of morbidity and suffering for the humankind[3]. Neurodegenerative diseases have long been defined by the properties of the neuropathological lesion observed in the brain[4]. Parkinson's disease (PD), the second most common progressive neurodegenerative disorder, is characterized by degeneration of dopaminergic neurons in the nigrostriatal pathway and subsequent dopamine (DA) depletion in the striatum[5]. Approximately 1 % of the population over the age of 65 and 4-5 % of the population by the age of 85 suffer from the most common form of motor system degeneration and the second most common neurodegenerative disorder, Parkinson's disease (PD)[6]. The etiology of PD is incompletely understood. Epidermiological studies and experimental models have suggested a role for both environmental exposures and genetic variability in PD pathogenesis. Complex I abnormalities, due to genetic mutation or polymorphisms, or man-made or natural substances, may play a pivotal role in the pathogenesis of PD by elevating oxidative stress and rendering cells vulnerable to excitotoxic insults[7]. Although various mechanisms of neuronal degeneration in PD have been proposed, the exact pathogenesis of PD is not well understood[8,9]. Increasing studies have demonstrated that one of etiologies about PD is the imbalance between free radical formation and the maintenance of the dopaminergic neuronal integrity through the endogenous antioxidant defense system[10,11]. Antioxidants, in the endogenous antioxidant defense system, are thought to play a major role in protecting brain against oxidative damage. It has been reported that the level of plasma in PD patient is significantly lower than that in healthy control subject[12].

Acorus calamus Linn. (Commonly called as 'Sweet flag') of family Araceae, is a semi-aquatic, perennial, aromatic herb with creeping rhizomes, sword shaped leaves and spadix inflorescence. *A. calamus* grows either as wild or cultivated crop throughout India ascending upto 1800 m in the Himalayas[13]. *Acorus calamus* is commonly known as ''bach'' or''ugragandha'' in India. Roots, rhizomes and leaves have been used in the Indian systems of traditional medicine for hundreds of years. *Acorus calamus* is a perennial herb with long, cylindrical rhizomes which are creeping and extensively branched which are up to 2.5 cm thick, which is white internally and brownish externally. The plant has long leaves which are about 1cm long and have a single prominent mid vein and on both sides slightly raised secondary and tertiary veins. The margin is curly edged or undulate. Plants very rarely flower or set fruit, but when they do, it consists of a leaf-like spathe and a spike-like spadix that is densely covered with yellow and green flowers which are 3 to 8 cm long, cylindrical in shape. The spadix, at the time of expansion, can reach a length between 4.9 and 8.9 cm. the inflorescence starts from early to late summer depending on the latitude, grows wild in marshy places up to 2000 m altitude in the Himalayas, Manipur, Naga Hills and in some parts of South India. From a karyotypic point of view, sweet flag includes four cytotypes: diploid [2x = 24], triploid [3x = 36], tetraploid [4x = 48] and hexaploid [6x = 72][14]. The plant has a characteristic essential oil called as the asarone oil. The composition of each chemical compound in the oil varies according to the ploidy level of the plant. It is believed that tetraploid plant has the highest beta asarone content [90-96%], triploid contains a small portion and diploid lacks it. Individual plants also show variation in the percentage of chemical components depending on the part of the plant from which the oil was extracted. Various compounds observed in different parts of *Acorus calamus* plant are listed out. Chemical isolation studies have led to the discovery that the two stereoisomers, α and β asarone, have psychoactive effects[15,16].

Traditionally *Acorus calamus* is used to treat appetite loss, bronchitis, chest pain, colic, cramps, diarrhea, digestive disorders, flatulence, gas, indigestion, nervous disorders, rheumatism, sedative, and vascular disorders. The plant has a long history from various countries and has been in use for at least around 2000 years in China and India[17]. It is widely employed in modern herbal medicine as its nueroprotective, sedative, laxative, diuretic and carminative properties. It is used in Ayurveda to counter the side effects of all hallucinogens. Roots, Rhizomes and Leaves of *A. calamus* have shown antioxidant, antimicrobial and insecticidal activities.*Acorus Calamus* was also known to many early American settlers and used for a number of diseases[17,18].

Materials & Methods :

Animals :

Male Sprague-Dawley rats weighting 200–250 g , were used for the study. Tha rats were inbred in the central animal house of the Department of Pharmacology, Karavali College of Pharmacy, Mangalore, and kept with a 12 hL : 12 hD light/dark cycle. Rats were housed in cages with food, water freely available & under suitable conditions of housing, temperature, ventilation and nutrition.

Plant Materials :

Collection of Rhizomes:

The dried rhizome of *Acorus calamus* was collected from local market of Jaipur in the month of October. It is

preserved in the departmental library for future reference.

Drugs and Chemicals :

Rhizomes were washed 2 or 3 times with tap water so that it was made free from all dust materials. They were cut into small pieces and dried under shade till they were brittle. The dried pieces of wood were powdered with the help of mixer grinder and 100g of powder is used for extraction. Steam distillation is a process employed to extract essential oils from organic plants by passing steam generated through the plant material. 6-OHDA and Apomorphine were purchased from Sigma Chemicals (St. Louis, MO, USA). 6-OHDA was dissolved in saline containing 0.1% ascorbic acid and prepared freshly in dark to avoid autooxidation. Store protected from light up to 12 months at −20°C. Apomorphine was dissolved in saline[19].

Animal Grouping and *Acorus Calamus* Treatment

The animals were divided into 3 groups (6 rats in each group): Control Group, 6-OHDA-lesioned group, AC-treated groups (250, 500, and 750 mg/kg). AC was dissolved in 5% tween 80 & administered orally once daily for 3 weeks[20].

6-OHDA Lesion

The left medial forebrain bundle (MFB) was lesioned by 6-OHDA. The rats were anesthetized with chloral hydrate (350 mg/kg, i.p.) and fixed in a stereotaxic instrument. Lesions were made by injecting 6-OHDA (20 µg in saline containing 0.1% AA) into the left MFB at the coordinate: , −2.5; , −2.0; , −8.5 mm, from bregma using a 5 µL Hamilton syringe at a rate of 1 µL/min. The Control Group animals were injected vehicle only (0.1% AA-saline) at the same coordinate. After injection, the syringe was left in place for an additional 5 min before being slowly retracted. 6-OHDA and sham rats were housed one week for further experiments[21].

Behavioral Studies :

Rotarod

Motor performance was also analyzed using the rotarod test that is a useful drug-free procedure for evaluation of motor abilities in rat models of PD[22,23]. Rotarod was used for the study of muscular coordination. It consisted of a rotating rod, 75 mm in diameter, which was divided into four parts by compartmentalization to permit the testing of four rats at a time. The time for each rat to remain on the rotating rod was recorded. The speed was set at 10 cycles per min and cut-off time was 180 s. The animals of all groups were trained on rotarod prior to the start of the experiment until they could stay on it at least for the cut-off time. After 1 week of lesioning, the rats of each group were tested on rotarod[24].

Narrow Beam Test

After 1 week of lesioning, the rats were tested for the balance and motor coordination on a narrow beam. The narrow beam had a smooth wooden narrow beam of 105 cm in length, 4 cm in width, and thickness of 3 cm. The beam was elevated from the ground by 1 m with additional supports. It had a goal box at the end of the narrow beam. There was food in the goal box for the reward of the animals. The rats were trained on the narrow beam for 10 trials per day with 1 min interval. The animals were allowed to explore the narrow beam for 10 trials with 1 min interval on 1 day before the experimental day and during this exploration rats were motivated and rewarded with food pellets in the goal box. The time to cross the beam was calculated[25].

Apomorphine-Induced Circling Behavior

Apomorphine-induced rotational behavior is a good indicator of extensive lesions of the nigrostriatal pathway[26], and is considered a valuable behavioral index to test novel drugs against PD[27]. The behavior was assessed by monitoring body rotations induced by intraperitoneal injection (i.p.) of apomorphine (0.5 mg/kg). The number of contralateral rotations was recorded for 30 min[28].

Neurochemical Assay :

Dopamine (DA) and its metabolites measurements by HPLC

Three days after the final 6-OHDA treatment, 3 rats from each group were sacrificed and their brains were quickly removed and placed on ice. Their striata from each animal were isolated, weighed and suspended in 0.5 ml of 0.2N perchloric acid. Each sample was sonicated and centrifuged at 11,000 x*g* for 15 min at 4°C. The supernatant was filtered through a 4-mm nylon syringe filter with a pore size of 0.45 µm. An aliquot of the filtrate was injected into a high performance liquid chromatography equipped with a C_{18} reverse phase, 3 µ LUNA column (100 mm × 2.0 mm). The sample was eluted by a mobile phase made of NaH_2PO_4 (25 mM), Na-citrate (50 mM), EDTA (0.03 mM), diethylamine HCl (10 mM), and sodium octyl sulfate (2.2 mM), at pH of 3.2, plus methanol (30 ml/l) and dimethylacetamide (22 ml/l) at a flow rate of 0.4 ml/min. The contents of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed by HPLC with electrochemical detection (ECD)[29].

Tyrosine hydroxylase (TH) immunohistochemistry

The rats were anesthetized with chloral hydrate (350 mg/kg, i.p.) and perfused transcardially through ascending aorta with 0.1 M phosphate buffer saline (PBS) at pH 7.4 followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brain was removed immediately and fixed in the same fixative for an additional 24 h at

4°C; furthermore the tissues were preserved in 10%, 20%, and 30% sucrose solution (in PBS) until they sank. The fixed tissues were embedded in OCT compound (polyvinyl glycol, polyvinyl alcohol, and water) and frozen at −20°C. Coronal sections of 20 µm thicknesses were cut on the cryostat. The sections were rinsed three times for 5 min in PBS, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol and incubated for 20 min at room temperature. Slices were permeabilized and blocked with PBS containing 1% Triton X-100 and 4% normal goat serum, for 1 h at 37°C. Thereafter, the sections were incubated in primary antibody (anti-TH mouse, 1 : 1000) for 24 h at 4°C, rinsed three times for 5 min in PBS, and subsequently incubated with avidin-biotin-horseradish peroxidase conjugate (ABC) for 1 h. After washing, the slides were incubated with biotinylated rabbit anti-mouse secondary antibody. The color was developed using DAB as a chromogen. Finally, sections were dehydrated in graded alcohol solutions, cleared in xylene, and coverslipped to be viewed under a microscope and photo micrographs were taken using a light microscope. Three sections per animal from the substantia nigra were selected. The intensity of the TH immunoreactivity at ×400 magnification was measured by semiquantitative densitometric analysis using an image-analysis program[30].

Western blotting

The substantia nigra (SN) of rat were dissected 3 d after the final 6-OHDA treatment. Protein in the SN was obtained and assayed. Antibodies directed against DJ-1 [1:6000] or α-synuclein (1:600) were used as the primary antibody. Horseradish peroxidase-conjugated IgG (anti-mouse or rabbit) was used as a secondary antibody. To ensure equal protein loading, membranes were stripped and immunostained for actin using an anti-β-actin antibody (1:1000) as the primary antibody. The band intensities were quantified using Quantity One software. Western blotting was repeated 3 times for each sample, with duplicate measurements for each blot[31].

Statistical Analysis

All values are expressed as mean ± S.E.M. Statistical analysis was performed out using a Student's *t* test comparing two groups and by using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test for the multiple drug-treated groups versus the control group. $P < 0.05$ was considered to be significant.

Results :

Behavioral Studies :

The Rotarod test

The rotarod test was used for the evaluation of muscular coordination in the present study. A significant depletion in muscular coordination in 6-OHDA-lesioned group was observed as compared with control group. AC (250, 500, and 750 mg/kg) was found to be effective in recovery of muscular coordination in a dose-dependent manner.

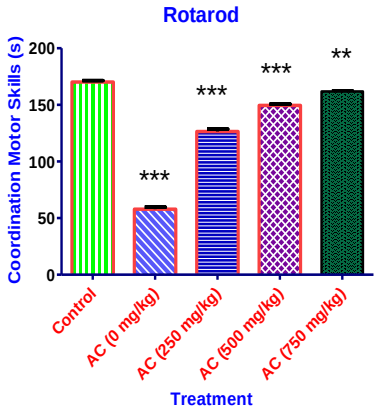


Fig.1.The Effect of AC on the Muscular Coordination
Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test.
*P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Narrow Beam Test

In the present study, the rats were tested for the balance and motor coordination on the narrow beam. The time taken to cross the beam was significantly increased in 6-OHDA-lesioned rats when compared with the control group. AC (250, 500, and 750 mg/kg) markedly decreased the time taken to cross the beam in a dose-dependent manner, indicating that AC showed significant improvement in balance ability.

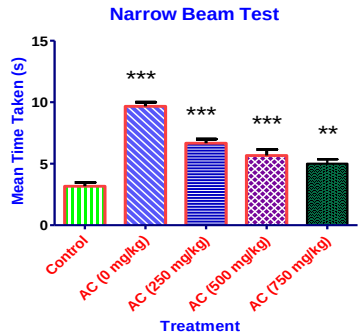


Fig.2. The Effect of AC on the Motor Coordination
Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test.
*P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Apomorphine-Induced Circling Behavior

Rotation induced by apomorphine was usually used to behaviorally assay the extent of neuronal loss following lesion by 6-OHDA. No rotation was observed in all rats in control group. Significant increases in the number of apomorphine-induced rotations were observed in the 6-OHDA-lesioned rats compared to the control group. As shown in Figure, rats receiving AC (250, 500 and 750 mg/kg) exhibited significant attenuation in circling behavior, indicating that AC significantly reversed this abnormal motor behavior of 6-OHDA.

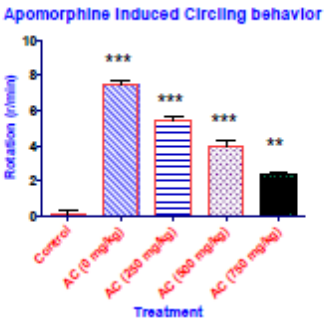


Fig.3. The Effect of AC on the Rotation Induced by Apomorphine
Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test.
*P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Neurochemical Assay :

Dopamine (DA) and its metabolites measurements by HPLC

The effects of AC on the levels of DA and its metabolites in the striatum of 6-OHDA induced PD model rat are shown in the Figure. DA, DOPAC and HVA contents in the striatum of 6-OHDA-lesioned rats were significantly lower than those in the control group (*P < 0.05, **P < 0.01, ***P < 0.001 vs. control group, respectively). After administration of AC, the striatal DA and HVA content of the AC group was higher than the 6-OHDA lesion group (*P < 0.05, **P < 0.01, ***P < 0.001 vs. 6-OHDA lesion group, respectively), and the striatal DOPAC content exhibited no statistically significant increase.

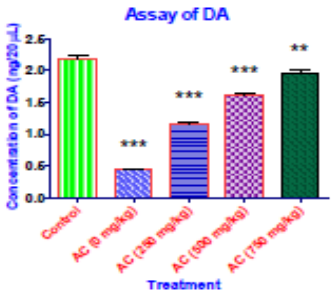
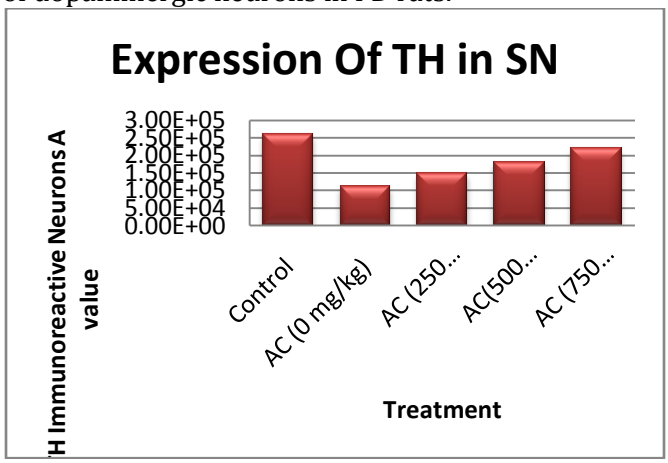


Fig.4. The Effect of AC on the Level of Extracellular DA in Striatum
Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test.
*P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Tyrosine hydroxylase (TH) immunohistochemistry

Immunohistochemical analysis showed that a marked depletion in the expression of TH in the SN in 6-OHDA-lesioned group compared to the control group and a pronounced restoration were observed in the AC-treated groups (250, 500 and 750 mg/kg) in a dose-dependent manner. AC decreased the loss of TH immunoreactivity compared with 6-OHDA-lesioned group. The result showed that AC increased the number of dopaminergic neurons in PD rats.



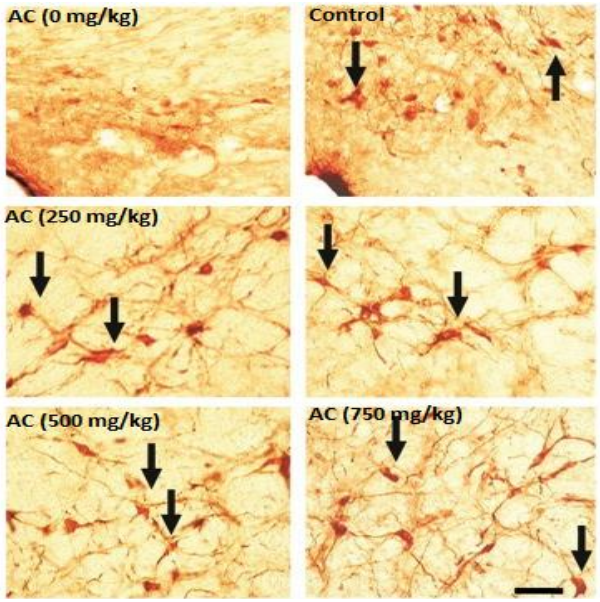
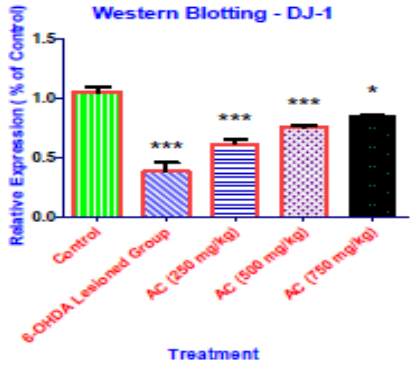


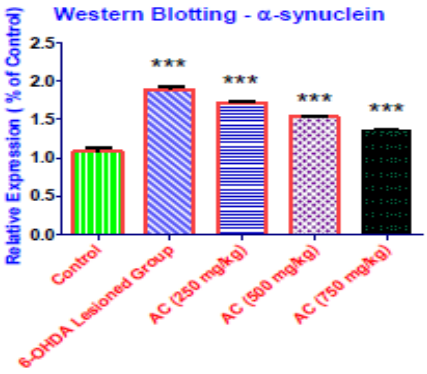
Fig.5. The Effect of AC on the Expression of TH in SN
Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test.
*P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Western blotting

Both DJ-1 and α-synuclein play important roles in PD and have altered expression levels in 6-OHDA lesioned animals[32]. Furthermore, there is evidence that DJ-1 and α-synuclein are closely related[33]. The expression levels of DJ-1 and α-synuclein in the SN of the 6-OHDA lesioned rats were measured using Western blot analysis. The level of DJ-1 in 6-OHDA lesioned rats was significantly reduced ($P < 0.01$, compared with control

group), leading to an upregulation of α-synuclein ($P < 0.05$, compared with control group). The administration of AC was able to prevent these changes. After administration of a high dose of AC (750 mg/kg), the levels of DJ-1 in the SN were significantly higher than in the 6-OHDA lesioned model group ($P < 0.05$, compared with 6-OHDA lesioned model group). Consistently, in the SN of the rats pretreated with various concentrations of AC, the levels of α-synuclein were lower than in the 6-OHDA lesioned model group (all $P < 0.05$, compared with 6-OHDA lesioned model group).





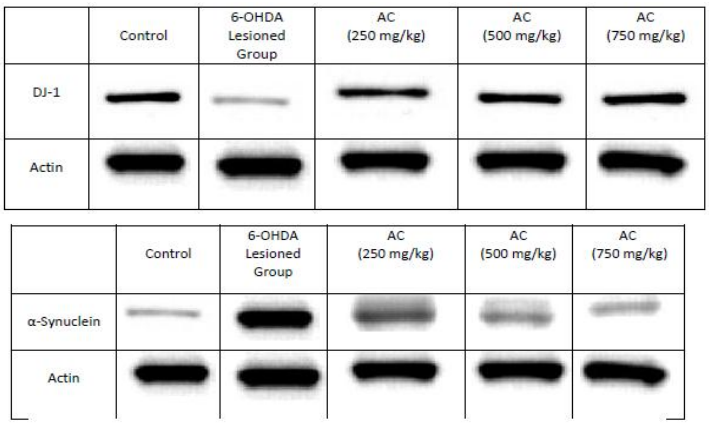


Fig.6. The expression of DJ-1 and α-synuclein in the SN of PD Rat model

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test.

*P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Discussion :

The results of this study clearly showed that the protective effect of *Acorus Calamus* on a rat model of PD is induced by 6-OHDA. It was found that treatment with AC for three weeks improved performances in three kinds of behavior test, increased the level of extracellular DA in striatum, and inhibited the loss of DA neuron in SN. Because 6-OHDA can be easily carried inside the dopaminergic neurons by the dopamine transporter (DAT), it is thought to be one of the most common neurotoxins used in degeneration models of the nigrostriatal dopaminergic system, in vivo and in vitro[34,35]. In this study, we used unilateral injection of 6-OHDA into MFB to establish a rat model of PD which mimicked behavioral, biochemical, and histopathological abnormalities observed in patients with PD[36].

In the present study, treatment with AC at all 3 doses for 3 weeks significantly improved the abnormal behaviors in PD rats induced by 6-OHDA, including the locomotion, muscle and motor coordination, and contralateral rotation induced by apomorphine. Some of them even almost recovered to the levels of sham rats. Our results suggested that AC had potent anti-Parkinson property.

It is suggested that AC possibly has anti-Parkinson property through improving the function of dopaminergic nerve. In the present study, AC was found to increase significantly striatal extracellular DA level and the expression of TH in SN in PD rats. Tyrosine hydroxylase is usually thought to be a rate-limiting enzyme in the synthesis of DA, and its expression is the marker for the DA neuron survival. Combined with previous studies and our present study, it is suggested that AC may have the neuroprotective action on PD by improving degeneration of dopaminergic neurons in the SN and DA depletion in the striatum.

To further investigate the mechanisms underlying the protective actions of AC, we studied the effects of AC on expression of DJ-1 and α-synuclein in the SN of a PD rat model by Western blot analysis. 6-OHDA treatment decreased the expression level of DJ-1 in the striatum of the rat. Overexpression of DJ-1 renders dopaminergic neurons resistant to neurotoxic insults. Drugs that enhance DJ-1 gene expression are believed to neuroprotective for PD[37]. In the present study, similar changes were observed in the SN in the 6-OHDA lesioned rat model. Our results showed that the expression of DJ-1 in the SN was decreased after 6-OHDA treatment, but pre-treatment with AC increased the level of DJ-1. Therefore, these results again suggest that DJ-1 plays a key role in the neuroprotective effect of AC. It is very likely that AC increases DJ-1 levels in the 6-OHDA lesioned rat model through direct interaction and

stabilization of DJ-1. However, other mechanisms, such as transcriptional upregulation, are also possible. α-Synuclein is the main structural component of Lewy bodies that is characteristic of both sporadic and familial PD[38]. It has been reported that α-synuclein in SN dopaminergic neurons is up-regulated following administration of 6-OHDA[39]. Consistent with previous studies, we found that the expression of α-synuclein in the SN was increased in 5-OHDA lesioned rat, but pre-treatment with AC markedly decreased the α-synuclein levels. These results indicate that α-synuclein might also be involved in the neuroprotective effects of AC. In addition, it has been reported that overexpression of DJ-1 could inhibit mutant human α-synuclein protein aggregation[40,41], and DJ-1 inactivation may promote α-synuclein aggregation and the related toxicity[42]. Whether the reduction in α-synuclein induced by AC is mediated by increased DJ-1 still needs to be explored further. In this paper, we focused on the anti-PD pharmacological effect of AC, thus we only observed its influence on DJ-1 and α-synuclein expression at a macroscopic level. Although the mechanistic study of AC is still preliminary and further investigations are required, this manuscript provides prospects for its future development.

The rhizome is the source of an essential oil, which is a unique source of oxygenated sesquiterpenes of great structural variety and responsible for significant antibacterial, antifungal, and insecticidal properties[43]. Apart from the terpenes, a few commonly occurring steroids and xanthones had also been reported. It was also used as a constituent of polyherbal formulation, namely herbal multiaction skin gel, which was found to be effective against a variety of specific and nonspecific dermatitis and maggot wounds[44]. The alcoholic extract of A. calamus rhizome exhibited potent antiviral activity against herpes viruses, that is HSV-1 and HSV-2[45]. The ethanolic extract of the rhizome is used as antiulcer agent that inhibits gastric secretion and protects gastroduodenal mucosa against the injuries caused by pyloric ligation in rats[46]. β-asarone isolated from the volatile fraction of A. calamus was found to exhibit anticarcinogenic action on ED_{50} of SGC cells at a dose level of 25 mcg/ml[47].

New pharmacological studies have almost confirmed the traditional uses of sweet flag as Nootropic Activity, Anti-diabetic Activity, Anti-seizures Activity, Antidepressant Activity, Neuromodulatory Effect, Anti cancer Activity, Anti-Oxidant Activity, Anti-Hypertensive Effect, Anti-HIV Activity[48], Cytotoxic Effect, Immunosuppressive Activity, Radioprotection and DNA Repair Activity[49], Coronary Vasodilation Effect, Anti-spasmodic and Anti-Diarrhoeal Effect, Insulin Sensitizing Activity[50], Wound Healing Activity, Anti-Inflammatory Activity, Synergistic Anthelmintic Activity, Anti-Hepatotoxic Activities, Anti-Ischemic Heart Disease Activitty, Anti-Fungal Activity, Anti-Bacterial Activity, Analgesic Activity, Anti-Pyretic Activity, Bronchodilatory Activity[51,52].

Major chemical constituents identified in sweet flag are α- and β-asarones along with other constituents, other constituents such as caryophyllene, isoasarone, methyl isoeugenol, and safrol are also responsible for medicinal activity but most of the biological actions of sweet flag have been attributed to presence of α- and β-asarones. Some chemical constituents of sweet flag β-asarone in particular, have been demonstrated to possess toxic effects like prolonged vomiting, hallucinogen, carcinogenic, and genotoxic action in dose dependent manner. Thus, low level of β-asarone could only be acceptable for therapeutic use, and the level of β-asarone can be minimized by decoction process. It appears to work mostly through a particular bioactive known as β-asarone, and this component appears to be effective in preclinical studies on the treatment of neuropathic pain & other neurological diseases. Previous studies shows that, It also exerts neuroprotective effect against middle cerebral artery occlusion-induced ischaemia in rat[53].

Recent evidence indicates that asarone improve degeneration of dopaminergic neurons in the SN and DA depletion in the striatum, thus it has neuroprotective activity against 6-OHDA induced PD in rats. As *Acorus calamus* has been successfully used in many health problems since a long time it provides a wide area of interest for the research purposes in development of newer drug molecules. The therapeutic potential should also be seen in combination with other medicinal agents. It has also been studied extensively from the point of phytochemical and pharmacological aspects which lead to the interest particularly in the area of Neuroprotective, antioxidant, antimicrobial and hypoglycemic actions. In recent years it has been noticed that more emphasis of research is on traditional medicines which have proven its authentification against treating various diseases. Various traditional uses are also known to be possessed by the plant like in rheumatism, bronchial asthma, leprosy, as cardiotonic and many more. Many activities are not studied till date and needs attention to explore further medicinal properties of the plant. Experiments will have to be conducted in future to exploit the full potential activities of this crop and this plant species has to properly identified and conserved to avoid the extinct condition.

Conclusion

The phytochemical screening of *picralima nitida* seed exhibit the presence of alkaloids, saponins, glycoside,

flavonoid, steroid and Anthraquinones and brine shrimp lethality assay shows LC_{50} of Aqueous, Methanol, Ethylacetate and Hexane extracts were 317ppm, 317ppm, 110ppm and 29ppm respectively, from this result, it is evident that the seed *picralima nitida staph.* may have curative properties against several human pathogens and suggest its importance in traditional medicine of tropical region of Africa. For further studies, effort should be devoted to characterising the bioactive components through activity-specific assays.

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