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Pharmacological assessments of polyphenolic extract of *Cymbopogon citratus* leaves in rodent model of parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is amongst the most common age-related neurodegenerative disorders. Natural compounds, especially the polyphenols have gained great interest as potential therapeutic agents in recent research. Thus, the present study was designed to evaluate the effect of polyphenolic extract of *Cymbopogon citratus (C. Citratus)* leaves in animal models of PD. In the given study PD was induced by administration of reserpine (5 mg/kg/day, i.p for 5 consecutive days), haloperidol (1 mg/kg, i.p.), in experimental animals. The symptoms of PD such as tremors, akinesia, rigidity and catalepsy were evaluated. Ethanolic extract of Cymbopogon citratus (CC) in doses of 100, 200 & 400mg/kg were administered per oral (PO). The L-dopa and carbidopa (30 mg/kg, PO) combination was used as a positive standard drug. Behavioural studies such as locomotor activity were performed. In catalepsy model, there is significant reduction in catalepsy duration in CC (100, 200, 400mg/kg) treated group as compared to the haloperidol group. In reserpine model, there is significant decrease of muscular rigidity, tremors, and akinesia in groups treated with CC (100, 200, 400mg/kg) dose dependently. Thus, the present study suggests the beneficial role of *C. citratus* leaves polyphenolic extract in treatment of parkinsonian like symptoms.

Keywords: Parkinson's disease; catalepsy test; haloperidol; reserpine, rotarod test, polyphenols, *Cymbopogon citratus*

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INTRODUCTION

In Parkinson's disease (PD) is a neurodegenerative disorder characterised by loss of dopaminergic neurons in the pars compacta of the substantia nigra (SN) results in parkinsonian syndrome[1]. It includes symptoms like bradykinesia, resting tremor, rigidity, and impairment of posture and gait[2]. Oxidative stress is thought to play an important role in dopaminergic neurotoxicity, mitochondrial dysfunction and neuroinflammation. Hence, there is a need of maintaining balance between oxidative stress and antioxidant system is necessary to preserve the structural integrity and optimal functions of brain[3].

Plant extracts and their constituents act as a natural source of antioxidants. The antioxidant activity of several plant extracts is due to several secondary metabolites especially phenolic compounds[4]. In recent times, many evidences support that the chronic consumption of polyphenols rich diet can promotes the healthy aging and prolongs the healthy life span by delaying the age-related disorders like PD. There are good evidences which suggest potential utility of polyphenols in treatment of PD[5].

Cymbopogon citratus (DC) Stapf (Poaceae), commonly known as lemongrass, is widely used in traditional medicine. The leaves mainly essential oils. Apart from this, leaves also constitute some other phytoconstituents including tannins, flavonoids, phenols, steroids, saponins, alkaloids etc. It is widely used in the food, pharmaceutical, cosmetic and perfumery industries for its essential oil. The literature survey indicates that *C. citratus,* possesses variety of pharmacological actions[6]. In addition to the use of volatile oil of the plant, its polyphenol rich extracts have been proven to have beneficial effects[7]. Apart from its therapeutic uses, *C. citratus* is also consumed as a tea, added to non-alcoholic beverages and baked food, and used as a flavouring and preservative in confections and cuisines. The aqueous extracts are also used in traditional medicine. Its infusion contains high levels of polyphenols, which are known for their antioxidant and anti-inflammatory properties[8]. However, in spite of reported antioxidant and neuroprotective activities, no major investigative reports found showing its role in Parkinson's disease.

Haloperidol and reserpine induced muscle rigidity models are important models, being comparatively simpler, is used extensively for examining potential symptomatic efficacy of new drugs in PD[9]. Thus, the current study envisages evaluation of the anti-parkinsonian effect of *C. citratus* in the management of PD. The study suggests that *C. citratus* leaves polyphenolic extract have potent antioxidant activity which

may reduce the degeneration of neurons associated with PD and reduces PD like symptoms.

MATERIALS AND METHODS

Plant material: The fresh leaves C. Citratus leaves were procured from verified supplier. The plant was identified and authenticated by Dr. Dongarwar, Assistant Professor, Department of Botany Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. A voucher specimen was deposited (Voucher Specimen No: 10037).

Animals: Sprague Dawley rats (150-200 g) of either sex were used. Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70%) with a 12:12 hr light: dark cycle. The animals were fed with standard pellet diet and water ad libitum. The Institutional Animal Ethical Committee approved the (IAEC Protocols no. PJLCP/2015/01)

Preparation of extracts: Shade dried leaves (100 g) were powdered and defatted with petroleum ether to remove volatile oils and further extracted with ethanol by using Soxhlet's apparatus. The ethanolic extract of C. Citratus leaves (CC) was concentrated under vacuum and evaporated to dryness and used for further studies.

Preliminary Phytochemical Studies: The screening for presence of phytoconstituents was carried out as per standard procedure described[10].

Total phenolic content determination: The total phenolic content of CC was determined by using the Folin–Ciocalteu assay[11]. Total phenolic content of BEPIF was expressed as mg Gallic Acid Equivalents. (GAE)/100 g dry weight. All samples were analysed in triplicate.

In vitro antioxidant activity (1, 1-diphenyl-2-picrylhydrazyl assay): DPPH radical scavenging activity of each plant extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Briefly, 3 ml of extract was added to 1ml of DPPH (2,2,diphenyl-1-picrylhydrazyl) solution (0.2 mM in methanol) as the free radical source. The mixture was shaken and kept for 30 min. at room temperature. The decrease of solution absorbance due to proton donating activity of components of each extract was determined at 517 nm. Lower the absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbic acid was used as reference standard. Percentage inhibition of DPPH free radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = [(Ac - A_E)/Ac] \times 100$$

Where Ac is the absorbance of the control, and A_E is the absorbance of the extract or reference standard. The antioxidant activity was expressed as IC50. The IC50 value was defined as the concentration in µg/ml of the extract that inhibits the formation of DPPH radicals by 50% [12].

Acute Oral Toxicity of the Extract: The mice were divided into 5 groups of 10 animals each. The mice were fasted for 6h and had access to only water ad libitum before experimental study. One group received only vehicle (distilled water). Other groups received different doses of CC at doses of 1000,2000,3000, and 4000mg/kg, respectively. All the doses and vehicle were administered orally. The animals were observed for 72h for mortality[13].

Experimental Design: Animals were randomly assigned into six groups; each group consisted of six animals (n = 6). Group I-vehicle. Group II-reserpine (5 mg/kg, i.p., for 5 consecutive days), or haloperidol (1 mg/kg, i.p.). PD was induced by administration of reserpine, haloperidol in animals. Group III-CC (100 mg/kg, p.o.). Group IV-CC (200 mg/kg, p.o.). Group V-CC (400 mg/kg, p.o.). Group VI-L-dopa + carbidopa (30 mg/kg, p.o.) were used for the treatment of Parkinson's symptoms.

Haloperidol induced catalepsy: Rats were divided into five groups of six each. They were pre-treated with vehicle, CC (100, 200 and 300 mg/kg, i.p.), and L-DOPA (30 mg/kg, i.p.) 30 min before haloperidol (1 mg/kg, i.p.). The duration of catalepsy was measured at 0, 30, 60, 90, 120, and 150 min after haloperidol administration using bar test. Both the forepaws of the animals were placed on a wooden bar elevated 9 cm above the ground. The cut off time (time for which animal was placed on elevated bar) was 300 seconds[14].

Reserpine-induced Parkinson Disease: For 5 consecutive days, animals were treated with reserpine at a dose of 5 mg/kg, i.p. After 24 h of last treatment animals were tested for induction of severity of tremors. The scores were assigned as follows: No tremors-0, occasional twitches-1, moderate or intermittent twitches-2, continues tremors-3. The number of tremors was counted for 5 min. If animals were not showing tremors then 0 score was given, if animals showed 1 or 2 tremors then 1 score was given, animals showed 3 or 5 tremors then 2 score was given and for 6 or more tremors, score was given 3. For evaluating akinesia animals were holded with the tail and putting the front paws on the platform and let the animal to walk while holding. The number of steps taken with forelimbs of animal were counted for 3 min. The muscular rigidity was determined by suspending the animal with forelimbs on middle part of horizontal glass rod (0.5 cm diameter) which was fixed at the height of 25 cm above the table top and time to fall on the bottom surface was measured. The cut-off was kept for 1 min. The animals were treated with CC (100, 200 and 400 mg/kg, p.o., respectively), or L-dopa-carbidopa (30 mg/kg, p.o.) 60 min before administration of reserpine for 5 consecutive days[15].

Locomotor Activity: After evaluation of tremors, akinesia, and muscular rigidity, locomotor activity was evaluated by using actophotometer. The apparatus consists of photoelectric cells, which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count was recorded for 10 min[16].

Statistical analysis: The statistical analysis was performed using Two-way analysis of variance and one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Comparisons were made between haloperidol or reserpine group and test/standard groups. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 5.0.

RESULTS

Qualitative phytochemical screening: The preliminary phytochemical screening of CC showed the presence of tannins and phenolic compounds.

Quantitative phytochemical estimation: Total phenolic content of CC was found to be 36.90 mg Gallic acid equivalent/100 g of dry weight.

Antioxidant activity: The IC50 value of CC by DPPH free radical scavenging method was found to be 16.30 µg/ml. The free radical scavenging activity of BEPIF was found to be comparable to ascorbic acid.

Acute oral toxicity: In acute oral toxicity studies, the CC was found to be safe at all the doses used and there was no mortality found upto the dose of 4000mg/kg. Therefore,

400mg/kg was used as the therapeutic dose and made variations by taking 100mg/kg as lower dose and 400mg/kg as higher dose.

Haloperidol induced catalepsy

Antiparkinsonian activity was studied using haloperidol induced catalepsy. In the present study, haloperidol produced maximum catalepsy after 90 minutes in vehicle treated animals. Pre-treatment with CC at different dose (100mg/kg, 200 mg/kg and 400 mg/kg, i.p.) showed a significant ($P < 0.05$, $P < 0.001$, $P < 0.001$ respectively) reduction in the duration of haloperidol induced catalepsy (Fig 1). Application of Two-Way ANOVA showed interaction between variables like treatment and time. Two way ANOVA followed by post hoc Dunnet test showed that, an decrease in cataleptic time was significantly [P<0.001, F (5, 180=1337.19)] reduced by all doses of CC after 30 min.

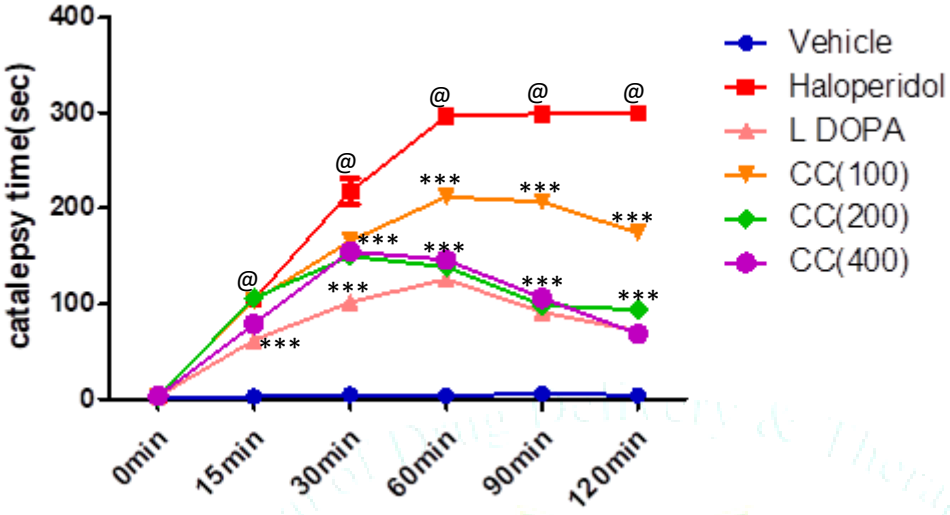

Figure 1. Effect of ethanolic extract of *C. Citratus* (CC) on catalepsy time in Haloperidol Model.

*Values are represented as mean ± SEM (n=6) Data was analyzed by Two-way ANOVA followed by Bonferroni multiple comparison test. @P<0.001 Considered as statistically significant as compared with Vehicle group. *P<0.05, **P<0.01***P<0.001 Considered as statistically significant as compared with haloperidol group.*

3.6 Reserpine Antagonism

Treatment with reserpine for 5 days produces muscular rigidity, tremors, and akinesia in animals. The intensity of muscular rigidity, numbers of tremors, and akinesia were significantly decreased by CC at 100, 200, and 400 mg/kg ($P < 0.05$, $P < 0.001$, $P < 0.001$ respectively) as compared with reserpine treated group. L-dopa-carbidopa treated group also showed a significant reversal of reserpine induced Parkinson's symptoms ($P < 0.001$). (see Figure 2, 3, 4)

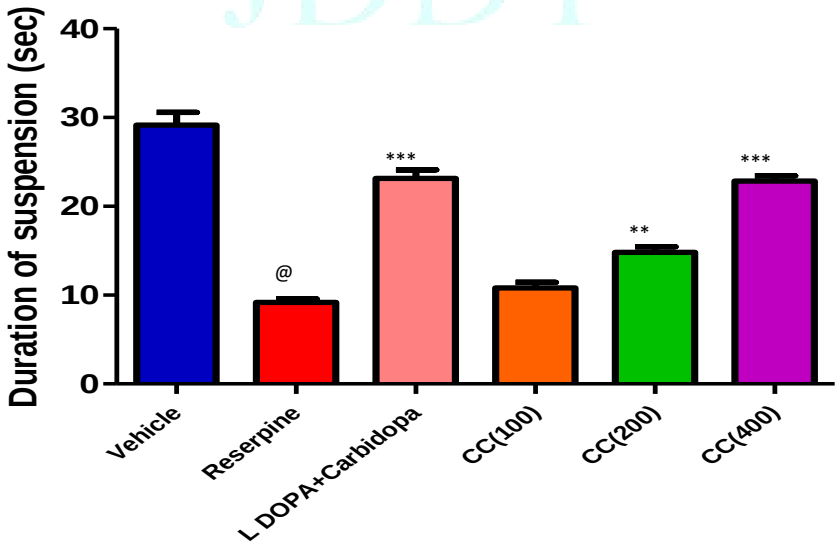

Figure 2. Effect of ethanolic extract of *C. Citratus* (CC)) on muscular rigidity in Reserpine model. *Values are represented as mean ± SEM (n=6) Data was analyzed by one-way ANOVA followed by Bonferroni multiple comparison test. @P<0.001 Considered as statistically significant as compared with Vehicle group. *P<0.05, **P<0.01***P<0.001 Considered as statistically significant as compared with haloperidol group.*

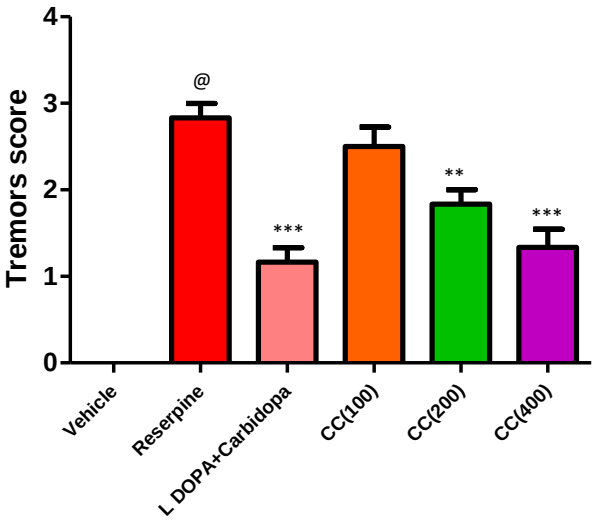

Figure 3. Effect of ethanolic extract of *C. Citratus* (CC) on tremors score in Reserpine model. *Values are represented as mean ± SEM (n=6) Data was analyzed by one-way ANOVA followed by Bonferroni multiple comparison test. @P<0.001 Considered as statistically significant as compared with Vehicle group. *P<0.05, **P<0.01***P<0.001 Considered as statistically significant as compared with haloperidol group.*

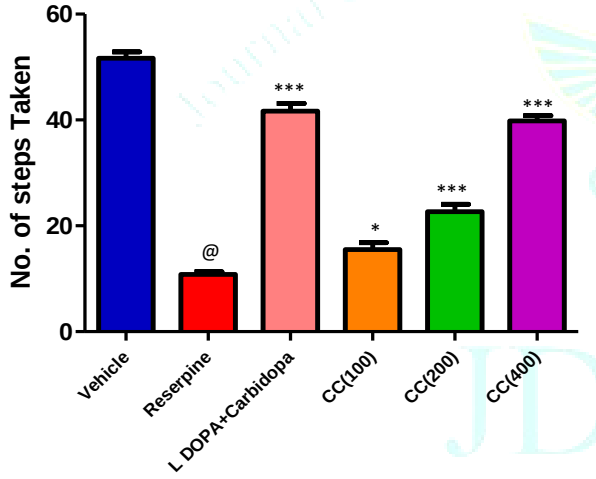

Figure 4. Effect of ethanolic extract of *C. Citratus* (CC) on akinesia in Reserpine model. *Values are represented as mean ± SEM (n=6) Data was analyzed by one-way ANOVA followed by Bonferroni multiple comparison test. @P<0.001 Considered as statistically significant as compared with Vehicle group. *P<0.05, **P<0.01***P<0.001 Considered as statistically significant as compared with haloperidol group.*

Thus, the results of haloperidol induced catalepsy and reserpine induced tremors, rigidity and akinesia, models confirmed the antiparkinsonian activity of CC.

3.7 Locomotor activity

The locomotor activity was significantly decreased by reserpine as compared to the vehicle group. The locomotor activity was significantly (P < 0.01) restored by CC at 100, 200, and 400 mg/kg and L-dopa-carbidopa (Figure 5).

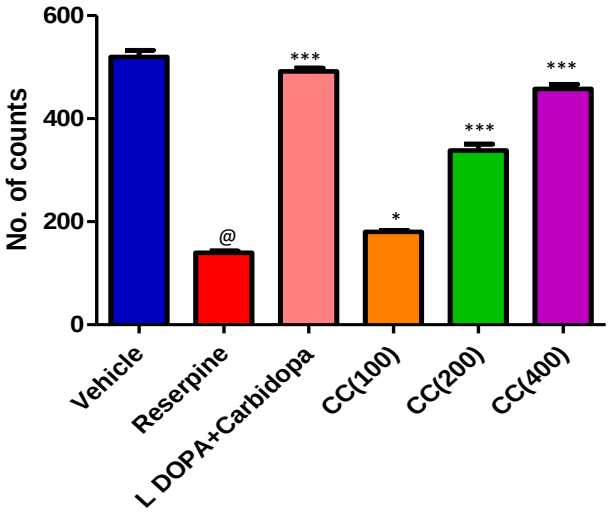

Figure 5. Effect of ethanolic extract of *C. Citratus* (CC) on Locomotor activity. *Values are represented as mean ± SEM (n=6) Data was analyzed by one-way ANOVA followed by Bonferroni multiple comparison test. @P<0.001 Considered as statistically significant as compared with Vehicle group. *P<0.05,**P<0.01***P<0.001 Considered as statistically significant as compared with haloperidol group.*

DISCUSSION

The present study demonstrates anti-Parkinson activity of *polyphenolic extract of C. Citratus* in different animal models like haloperidol and reserpine.

The present study, haloperidol (1 mg/kg) treated animals exhibits cataleptic behaviour similar to the symptoms of PD. Haloperidol works by antagonizing dopamine D2 and, to a lesser extent, D1 receptors in medium spiny neurons that comprise the indirect and direct pathways of the motor circuit respectively. The resultant block of striatal dopamine transmission results in abnormal downstream firing within the basal ganglia circuits that is manifest as symptoms of muscle rigidity and catalepsy within 60 min of haloperidol (0.5– 5 mg·kg-1, i.p.) injection[17]. Catalepsy is a behavioural state in animals such as mice and rats in which they fail to correct externally imposed postures which is not directly associated with PD. However, catalepsy may be likened to the inability of patients to initiate movements and so could be considered a worthwhile measure. Haloperidol-induced catalepsy is one of the animal models to test the extrapyramidal side effects of antipsychotic drugs. The agent, increasing dopamine transmission inhibits neuroleptic induced catalepsy. Research showed that acute administration of haloperidol reduces striatal content of dopamine, noradrenaline and 5-HT[18]. The ethanolic extract of c. citratus (100, 200 and 400 mg/kg, p.o.) showed a significant reduction in the duration of catalepsy demonstrating antiparkinsonian activity. The inhibition of catalepsy indicates the ability of the drug to potentiate dopaminergic transmission in the striatum.

Reserpine works by inhibiting the vesicular monoamine transporter (VMAT2). This leads to loss of storage capacity and hence depletion of brain (and peripheral) monoamines including noradrenaline and 5-HT as well as dopamine. It is reported that reserpine produces ~85% loss of dopamine in the SNpc and >95%dopamine depletion in the striatum within 2 hours of injection [19]. CC (200 and 400 mg/kg, p.o.) and the combination of L-dopa and carbidopa produced a significant reduction in these symptoms in rats. *C. Citratus*

was also able to increase the locomotor activity in rats. As reserpine induced motor defect was significantly reversed by *C. Citratus* which demonstrates its anti-Parkinson's activity.

The above behavioural results suggest that *C. Citratus* has the ability to improve symptoms of Parkinsonism, in part, by the restoring the level of dopamine, and by the regulation of the antioxidant system. Thus, antioxidant and neuroprotective activities may be responsible for antiparkinsonism effect. Hence, *C. Citratus* may be useful in the treatment of PD. The above observed beneficial effects of *C. Citratus* may be attributed due to diverse chemical components mainly polyphenols.

CONCLUSION

C. Citratus significantly reduced the symptoms of PD may be due to antioxidant and neuroprotective activities or increase in the level of brain dopamine similar to L-dopa and carbidopa. Thus, *C. Citratus* may have therapeutic potential in the treatment of PD.

Further, studies necessitate the estimation of brain dopamine level and isolate the individual constituents responsible for potential activity.

Acknowledgment: Nil

Conflict of Interest: None

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