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Evaluation of *C. cassia* Effectiveness in Behavioral Modulation of Haloperidol Induced Parkinson's Disease (Mice Model)

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAZ designed the study, wrote the protocol. Author TAK wrote the first draft of the manuscript. Authors LS and MAK supervise and improve the figures quality. Authors MY, AA and AS performed and managed the experimental process and author ZUR identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was undertaken to evaluate the effectiveness of behavioral modulation of *C. cassia* extract against Haloperidol induced Parkinson's disease (PD) in albino mice.
Study Design: The study used animal models to test the effect of Haloperidol in development of PD and effectiveness of *C. cassia* extract in behavioral modulation in mice before & after the treatment of PD.
Place and Duration of Study: Pharmacology Lab, Department of Pharmacology, Faculty of Pharmacy, Hajvery University, Lahore-Pakistan.

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Methodology: The study was divided into three phases; During Phase I, all subjects were randomly divided into four groups comprising of 7 subjects each through flip of coin method and were trained for wire hanging test, grip strength test, Vertical rod test, and swim test for seven days. During Phase II, PD was induced by intraperitoneally administering 1 mg / kg / day of Haloperidol for 7 days. Animals of Group A (Normal) was served as control, Group B (Diseased, HP) received haloperidol (1 mg/kg / day), Group C (HP+CN-100), and Group D (HP+CN-200) were administered *C. cassia* extract, 100 mg and 200 mg per kg of body weight orally for 36 days respectively. During Phase III the above mentioned test were performed and effects of *C. cassia* extracts were recorded. The results of the present study revealed that dose of *C. cassia* extract 100 mg/kg is more safe and effective than 200 mg/kg. However, in swim test Group D (HP+CIN-200) is more statistically significant as compared to Group B (Diseased, HP) with *P = .003*.
Conclusion: The current study concludes that oral administration of *C. cassia* extract modulates haloperidol induced behavioral changes in mice. The present study suggests that extract will be helpful as adjunct therapy along with standard therapy of Levodopa/Carbidopa.

Keywords: C. cassia; haloperidol; behavioral modulation; Parkinson's disease; mice.

1. INTRODUCTION

Parkinson's disease (PD) was first illustrated by James Parkinson in 1817 in "An Essay on the Shaking Palsy" [1]. The major symptoms of PD are tremors, bradykinesia, postural instability and rigidity, postural abnormalities, akinesia and festinating gait. These symptoms are led by psychological symptoms such as depression and more general non-motor symptoms such as olfactory dysfunction constipation, and sleep disturbances [2]. The incidence of Parkinson's disease (PD) is nearly about 1% at the age of 65 years which further increased to 5% with the age of 85 years [3]. Haloperidol is a typical neuroleptic drug and shows effect by blocking the postsynaptic dopamine D_2 receptors in the mesolimbic system and cause an escalation of dopamine turnover by blockage of the D_2 receptors [4].

Anticholinergic and β-adrenergic receptor blocking effects of haloperidol is quite weak. Parkinson disease is characterized mainly by the loss of melanin containing dopaminergic neurons in zona compacta of the substantia nigra [5]. Haloperidol cause a decrease in dopamine neurotransmission [6]. Clinical symptoms appear only when dopaminergic neuronal death exceeds a critical threshold 70-80% of striatal nerve terminals. The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines [7].

C. cassia essentially contains essential oils and different derivatives, for example, cinnamaldehyde, cinnamic acid, cinnamate, and various different components, for example, polyphenols. In addition to being an anti-oxidant, anti-inflammatory, antidiabetic, anti-cancer, anti-microbial, lipid-lowering, and cardiovascular-illness lowering agent, cinnamon has likewise been accounted for as helpful for metabolic disorder, increases insulin sensitivity, polycystic ovary disorder, expanding incline body mass, and gastric emptying. It may be valuable against neurological illness, for example, Parkinson's and Alzheimer's disease [8,9]. There are specific proteins present in nervous system called as tau protein. Tau is required for the usual structure and role of neurons in the cerebrum. *C.cassia* can shape neurofibrillary tangles, which are a distinctive character of Alzheimer's disease. Cinnamaldehyde and epicatechin were found to shield tau from oxidative stress that can prompt to dysfunction [10].

The objective of this study were to evaluate the effect of Haloperidol in development of PD, effectiveness of *C. cassia* extract to modulate behavioral activities in mice model PD and record the behavioral changes in mice before & after the treatment of PD with *C. cassia* extract.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Albino mice (20 to 25 g) were taken from animal house of Uvas (University of veterinary sciences). The animals were acclimatized and kept under specified temperature (22 ± 2°C) and humidity (60 ± 2%) under 12-hours light / dark cycles with food and water ad libitum. Experimental procedures & animal handling were approved by Institutional Committee of Research

Ethics, Hajvery University (Ethical no. 710EN-2015) Lahore - Pakistan.

2.2 Plant Material

The bark of *C. cassia* was obtained from botanical garden of Punjab University, Lahore Pakistan (Herb No. 718-2015) and Taxonomical identification and authentication of bark was done by department of botany Government College University, Lahore Pakistan.

2.3 Drugs

Haloperidol (source: Sigma-Aldrich, St. Louis, USA).

2.4 Experimental Design

The study was divided into three phases; During Phase I, the behavioral modeling of the experimental mice was performed. Animals of all groups were trained and divided into four groups comprising of 7 subjects each. During Phase II, PD was induced by administering Haloperidol of 1mg / kg per day intra-peritoneally for 7 days. All animals were observed for 30 minutes post injection and then after hourly intervals for next 3 hours. At the end of the 7th day, PD was assessed by hind limb movements and behavior [11]. After induction of disease, subjects were divided into four groups. Group A containing normal mice with i.p injection of saline served as control. Group B received intraperitoneal injection of haloperidol (1 mg/kg per day) for seven days. Group C and D were administered *C. cassia* extract, 100 mg and 200 mg per kg of body weight orally for 36 days respectively. During Phase III, following four tests were performed on daily basis to check the difference in the behavior before induction of PD and after treatment. These tests include wire hanging test, grip strength test, Vertical rod test, and swim test.

2.5 Statistical Analysis

Data was statistically analyzed on SPSS version 22.0 using ANOVA with a $P < 0.05$ considered as significant. Graph Pad Prism software was used to design the graphs.

3. RESULTS

3.1 Wire Hanging Test

Efficacy of treatment protocols was assessed using neuromuscular and sensory-motor function parameters. One of these parameters to assess neuromuscular strength is wire hanging test. It is performed to assess the neuromuscular ability of mice. It was observed during experimentation that animals of Group C showed more neuromuscular strength than Group D as shown in Fig. 1. However, results were not statistically much significant ($P < 0.05$) in comparison with results seen in Group D.

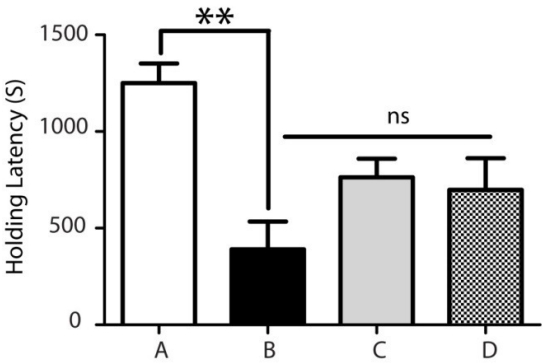

Fig. 1. Wire hanging test

3.2 Grip Strength Test

Another tool to measure treatment effectiveness is grip strength test. Grip strength test showed that mice of Group C had strong grip as compared to mice of Group D and Group B mice as shown in Fig. 2. Results were found statistically significant for Group C ($P<0.05$) in comparison with mice of Group D ($P<0.05$). It was also observed during study that mice of Group B (Diseased) had shown least strength of grip than mice of Group A ($P<0.01$) as demonstrated in Fig. 2.

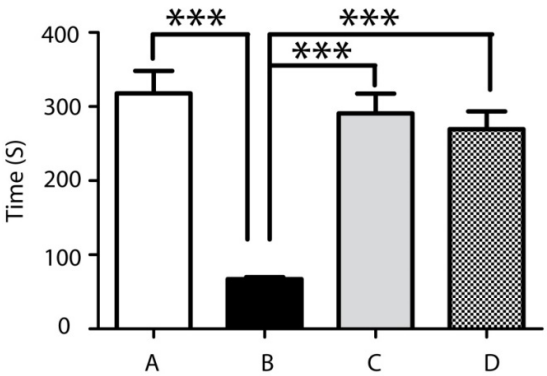

Fig. 2. Grip strength test

3.3 Vertical Rod Test

Vertical rod test is used to measure the sensorimotor function of mice. PD motor tests

provide a good read-out of neurological function. It was observed during experimentation that mice of Group C showed improvement in behavioral and sensorimotor function than mice of Group B ($P<0.05$) as shown in Fig. 3. In case of mice of Group D, it was seen that this group was statistically significant than mice of Group B ($P<0.05$) while it was also noted that this group also showed better sensorimotor function as compared to mice Group C ($P<0.05$). However, results were also found significant for mice of Group B as compared to mice of Group A ($P<0.01$).

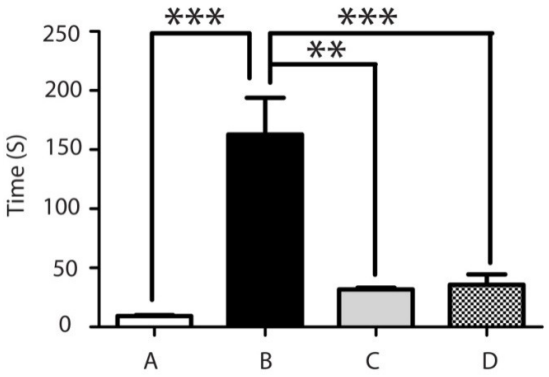

Fig. 3. Vertical rod test

3.4 Swim Test

In case of Swim Test, Group D is statistically significant in association with mice of Group C ($P<0.05$) and mice of Group B ($P<0.05$). Similarly, it was observed that mice Group A is also statistically significant than mice of Group B ($P<0.01$) as demonstrated in Fig. 4. Consequently in our study, it is observed that Cinnamon 100 mg / kg dose is found effective as compared to 200 mg / kg. However, swim test predicts vice versa.

4. DISCUSSION

Prevalence and incidence of PD is higher in males than in females [12]. The regimen of haloperidol (0.5–5 mg/kg, i.p) works by blocking dopamine D2 and, to a lesser degree, D1 receptors in medium spiny neurons that include the supplementary and direct pathways of motor circuitry individually. This outcomes in blockage of striatal dopamine causing atypical downstream changes inside of the basal ganglia which is visible as side effects of muscle inflexibility and catalepsy within 60 min of haloperidol (0.5– 5 mg/kg, i.p.) injection [13].

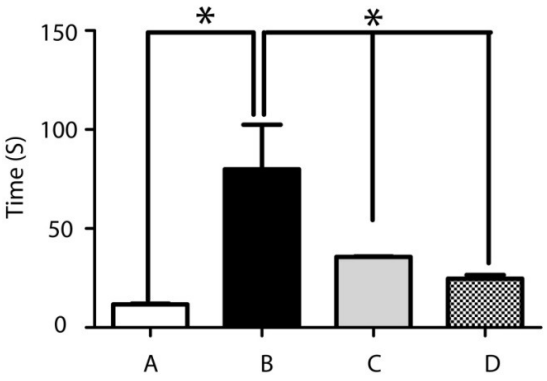

Fig. 4. Swim test

The main goal of the current study was to characterize the effects of Haloperidol in the development of PD models and behavioral tasks before, during, and after treatment with *C.cassia* extract. In the present study, chronic haloperidol treatment significantly increased vacuous chewing movement and tardive dyskinesia as compared to control animals. Neuroleptics act by blocking dopamine receptors [14]. Such blockage results in increased dopamine turnover, which in turn leads to increased production of hydrogen peroxide, resulting in oxidative stress [15]. Existing evidence indicates that excessive production of free radicals is associated with chronic neuroleptic use and might contribute to the onset of tardive dyskinesia and other movement disorders, such as dystonia and Parkinsonism [16].

The wire hanging tests can be used to assess global muscle function and coordination over time in young and old mice. The test is based on the latency of a mouse to fall-off a metal wire upon exhaustion. With the use of several described experimental designs, the natural course of the disease or the efficacy of genetic or pharmacologic treatment strategies can be assessed. It was observed during experimentation that mice of Group C showed more neuromuscular strength than those of Group D as shown in Fig. 1. However, results were not statistically much significant ($P>0.05$) in comparison with mice of Group D. This showed that although both doses did not improve much of the neuromuscular strength but these doses were found equally effective in improvement of muscle function and coordination. In one study where effect of *Hypericum hookeranium* (Ethanolic extract of *H. hookeranium*, EEHH) on Haloperidol induced neuromuscular weakness was tested by wire hang test. The less latency to

fall by releasing the wire soon indicated the apathetic state in the induced animal. The latency in falling represents the improved neuromuscular strength in 400 mg/kg EEHH treated animals with the same effect as that of the standard drug scopolamine. Before treatment animals had excellent neuromuscular activity, which was reduced by the treatment of Haloperidol, *H. hookeranium* at the dose 400 mg/kg [17]. Therefore, our study suggests that the effect of low doses of *C. cassia* extract 100 mg/kg & 200 mg/kg show more favorable outcome as compared to *H. hookeranium* at the doses of 400 mg/kg.

The grip strength test is a modest non-intrusive method intended to assess mouse muscle power in vivo, by taking benefit of the animal's affinity to grip a flat metal bar or framework while suspended by its tail or each of the four appendages. In our study, grip strength test showed that Group C mice had strong grip as compared to mice of Group D and Group B mice as shown in Fig. 2. Results were found statistically significant for mice of Group C ($P<0.05$) in comparison with mice of Group D ($P<0.05$). It was also observed during study that mice of Group B (toxic group) had shown least strength of grip than mice of Group A ($P<0.01$) as demonstrated in Fig. 2. The dose of 100mg/kg against haloperidol induced mice model of PD showed more promising effects during grip strength test as compared to 200 mg/kg in mice of Group D. In relation to our study, same tests were applied on mice model to check the effects of haloperidol on grip strength test. Haloperidol treated mice when subjected to motor integration tests such as grip strength test, showed a decrease in muscle coordination which could be due to a loss of muscular strength. Treatment with MECD (Methanolic Extract of *Canscora decussate*) showed a significant improvement in the muscle coordination as there was an increase in retention time and fall-off time in grip strength test respectively. [18]. Therefore, results reveal that 100 mg/kg dose shows increased grip strength and less fatigue in comparison to dose of 200 mg/kg of body weight (Fig. 2).

Vertical rod test is used to measure the sensorimotor function of mice. PD motor tests provide a good read-out of neurological function. In one of the studies on rod test, haloperidol (0.125, 0.25 and 0.5 mg/kg) produced the extension of T-turn and TLA as indicator of bradykinesia in mice and the elongation lasted at least 7 hr after haloperidol treatment. Intraperitoneal co-pretreatment with L-DOPA (400 mg/kg) + carbidopa (10 mg/kg) decreased the catalepsy induced by haloperidol at a dose of 0.125 mg/kg in mice, while co-pretreatment with L-DOPA (200 and 400 mg/kg) + carbidopa (10 mg/kg) dose-dependently decreased the haloperidol (0.125 mg/kg)-induced bradykinesia. The effect of L-DOPA + carbidopa in vertical rod test was more marked as compared to catalepsy test [19]. The treatment with *C.cassia* 100mg/kg & 200 mg/kg in our study showed significant results in mice model of PD. It was observed during experimentation that mice of Group C showed improvement in neurological function than Group B ($P<0.05$) as shown in Fig. 3. In case of animals of Group D, it was seen that this group was statistically significant than Group B ($P<0.05$) while it was also noted that this group also showed better neurological function as compared to Group C ($P<0.05$). However, results were also found significant for animals of Group B as compared to Group A ($P<0.01$). The data shows that the *C. cassia* (100 mg/kg) is more effective than dose of 200 mg/kg and in comparison to the above study it can be used as adjunct therapy or in comparative therapy with Levodopa/Carbidopa.

It has been described as rendering a situation in which "behavioral despair" is induced; that is, the animal loses hope to escape through the stressful environment. Swim test used to examine motor damage and measurement of motor weakness in animal models [20]. The swim test is used for the assessment of anti-depressant medications, upper viability of new drugs, and control trials that are used for blocking or counteracting depressive-like states. In this study on *C. cassia* extract, at the doses of 100 mg/kg and 200mg/kg in haloperidol induced mice model of PD, revealed results which are much more significant as compared to previous studies. However, 100 mg/kg dose shows more promising results as compared to 200mg/kg of extract. In our study, the data show more promising results in case of swim test of mice of Group D which is statistically significant in association with mice of Group C ($P<0.05$) and Group B mice ($P<0.05$). Similarly, it was observed that mice of Group A is also statistically significant than mice of Group B ($P<0.01$) as demonstrated in Fig. 4.

5. CONCLUSION

In the present study, the activities of mice were reduced after the administration of haloperidol

due to its effect on dopamine blockade and development of symptoms of PD and behavioral changes. Oral administration of *C. cassia* extract defends haloperidol prompted behavioral changes. The findings of the present study suggested that the use of *C. cassia* extract may be helpful alone as well as an adjunct therapy with standard therapy of Levodopa/Carbidopa in Parkinson's disease treatment and behavioral modulation.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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