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Effect of *Hypericum perforatum* extract on behavioural studies in experimentally induced neurodegenerative disease

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ABSTRACT

Parkinson's disease (PD) is a common neurodegenerative movement disease affecting a large number of people worldwide. In animals, the intracranial or systemical application of the neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) can result in severe injury to the nigrostriatal dopaminergic system. *Hypericum perforatum* is a plant generally used as an antidepressant that also has anti-inflammatory and antioxidant properties. *Hypericum perforatum* has been used to lessen the mild to moderate symptoms of depression. The present study was done to find the effect of *Hypericum perforatum* methanolic extract pretreatment and post-treatment on behavioral studies in MPTP induced Parkinson's disease model. The behavioral assessments were performed with a forced swim test, pole test, tail suspension test, and catalepsy test. *Hypericum perforatum* extract ameliorated depressive-like *behavior* better in post-treatment as compared with pretreatment group in MPTP model of *Parkinson's* disease. It can be said that *Hypericum perforatum* methanolic extract might be a therapeutic agent for the treatment of PD, but further clinical studies are required.

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INTRODUCTION

PD is a multisystem neurodegenerative disease in which progressive loss of midbrain dopamine neurons, with resulting dopaminergic deafferentation of the basal ganglia, excites characteristic motor instabilities that comprise muscular rigidity, resting tremor and slowing of movement. It affects 1% of the elderly population (Deng *et al.*, 2018). Clinically PD is characterized by postural tremor, bradykinesia, rigidity and instability along with psychiatric, autonomic and cognitive problems. Many neurotoxins used for PD model induction that leads to nigral cell degeneration with accentuating oxidative stress (De Pablo-Fernández *et al.*, 2019).

The neurotoxin "MPTP" became a priceless tool to make experimental parkinsonism, which is used as a model of PD. In individuals, MPTP generates dopaminergic nigrostriatal neurodegeneration of the basal ganglia, which is predominantly afflicted in Parkinson's disease (Kasahara *et al.*, 2017). This neurotoxin excites virtually all symptoms of the

idiopathic disorder, comprising rigidity, akinesia, gait/posture abnormalities and tremor (Yang *et al.*, 2017). In medical society, the treatment for Parkinsonism without adverse effects is required more for the people suffering from this disease.

Hypericum perforatum (Saint John's wort) was considered a traditional folk medicine used topically for treating wounds, burns, abrasions, sunburns and inflammatory skin disorders. Its use in wound healing could be justified with its antimicrobial, anti-inflammatory, and astringent effects. Hypericum oil can be used topically for treating haemorrhoids and burns, to decrease inflammation, and as an antiviral and anesthetic. *Hypericum perforatum* extracts are becoming one of the standard treatment for antidepressant therapy (Ng *et al.*, 2017). H. perforatum has various therapeutic potential, including anti-inflammatory and anxiolytic effects. H. perforatum extract has a beneficiary effect on chronic stress model in Hypericum extract has many components with well documented biological activity including naphthodianthrones (pseudohypericin and hypericin), a wide range of flavonoids (hyperoside, quercetin, and isoquercitrin) and the phloroglucinols (pseudohyperforin and hyperforin) (Cao *et al.*, 2017). With this evidence this plant was selected for research work to find out the efficacy of *Hypericum perforatum* on degenerative neuro diseases. Their present study was done to find the effectiveness of *Hypericum perforatum* methanolic extract (HPE) on behavioural studies in MPTP induced Parkinson disease.

Methodology

Animals

Three months old C57BL/6 male mice weighing 23g - 28g were used to carry out the experiments. The experiments were approved by IAEC, Adhiprasakthi College of Pharmacy (APCP/IAEC/2015-2016/4) and performed out in accordance with standard operating procedures in "guidelines on the regulation of scientific experiments on animals" (CPCSEA guidelines) by the ministry of environment and forest, Government of India. The animals were kept in polycarbonate cages in the standard day-night cycle and the temperature kept at 22 ± 2^0C. The mice were fed with Amruth Rat Feed, provided by Pranav Agro Industries (Pune, India) and had unrestricted access to water ad libitum.

Experimental induction of MPTP

The experimental induction of Parkinsonism to mice was done by giving an intraperitoneal (i.p) injection of MPTP hydrochloride (30 mg/kg b.w), dissolved in physiological saline for five consecutive days. Safety protections for the use of MPTP in chemical preparation and animal injections were taken by following the method of (Kasahara *et al.*, 2017).

Plant material and preparation

Hypericum perforatum plant was purchased from JK medicinal plants introduction center (JKMPIC), Srinagar, Jammu and Kashmir with authentication (no: JKMPIC-(K) R&D 20119). The air-dried sample was powdered finely using an auto-mix blender and kept in a deep freezer till use. The methanolic extract made using Soxhlet apparatus was concentrated by rotary evaporator at 40°C and kept in a cool place. These samples were evaporated to dryness and were dissolved in water for further behavioral studies.

Experimental grouping

The animals were randomly classified into 5 groups

Group 1- Control group, the mice received 1ml of distilled water orally.

Group 2- Mice were treated with *Hypericum perforatum* methanolic extract (HPE) 1ml orally as a single dose.

Group 3- Mice were administered with MPTP (30mg/kg, i.p) as a single dose for 5 consecutive days.

Group 4- Mice were administered with MPTP (30mg/kg, i.p) as a single dose for 5 consecutive days and post-treated orally with HPE (300mg/kg b.w) as a single dose.

Group 5- Mice pretreated orally with HPE (300mg/kg b.w) as a single dose and then injected with MPTP (30mg/kg, i.p) as a single dose for 5 successive days.

After the treatment period, behavioral assessments were performed with a swim test, tail suspension test, pole test and catalepsy test.

Behavioral Assessment

Forced swimming test

Forced swimming was carried out following the method of (Cao *et al.*, 2017). Briefly, a transparent acrylate cylinder (60 cm high; 24 cm diameter) was filled using 25°C water (25 cm deep). The mice swim for 5 consecutive days for 10 min daily and 4 weeks later on day 33. The total time period of the FST was fixed at 4 minutes (240 seconds). During behavioral analysis, the time each mice expend mobile is evaluated and noted. The complete mobility time is reduced from 240 seconds. This shows the immobility of time. This method was taken as it is better to note down the movements. Any movement other than those essential for stabilizing the body

and keep the head above the water was measured as mobility.

Tail suspension test

The tail suspension test was done according to the method of (Pałucha-Poniewiera *et al.*, 2017). The tail (2 cm from the end of the tail) of the animal was hanged upward down using adhesive tape. An animal was arbitrated to be immobile when it stopped moving limbs and body, making only movements allowing to breathe and the total period of immobility in seconds was measured.

Pole Test

Animals were kept head-up on top of a vertical wooden pole. The pole base is kept in a cage having a bed and when kept on the pole, mice orient themselves downward and incline the length of the pole back into the cage. Animals received five test trials after two days of training. The times to orient downward and total duration to incline are measured (Yao and Zhao, 2018).

Catalepsy test

The hind limbs of mice were kept on a wooden block (3 cm high), and mice latency in moving to the smooth surface was examined (Bhattacharjee *et al.*, 2016).

Statistical analysis

All the results were measured as Mean ± SEM of a number of experiments. The statistical variation was observed by one-way analysis of variance (ANOVA) with the help of SPSS version 11.5 (SPSS, Cary, NC, USA) and the intercomparison between the groups was attained by Duncan's Multiple Range Test (DMRT). A P value less than 0.05 was measured to indicate a significant variation between groups.

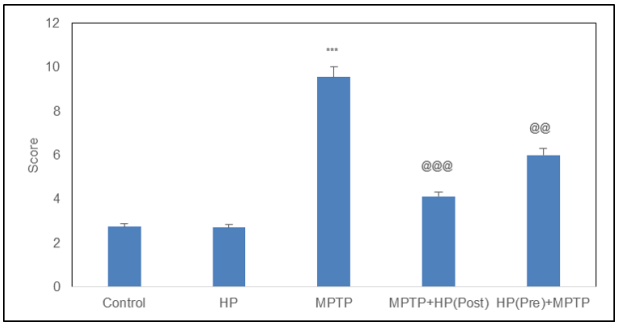

Figure 1: Effect of Hypericum perforatum on behavioural studies using swim test score. Results are measured as Mean ± SEM (n=6)

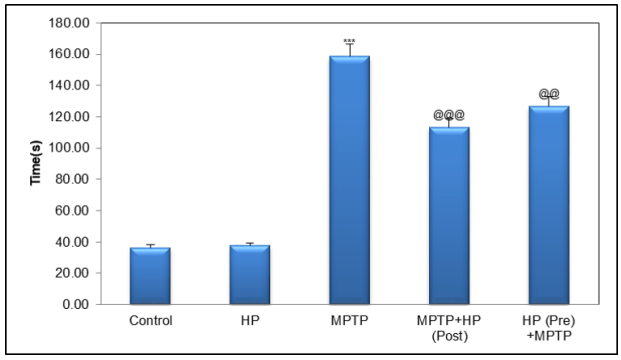

Figure 2: Effect of Hypericum perforatum on behavioural studies using tailsuspension test score. Results are measured as Mean ± SEM (n=6)

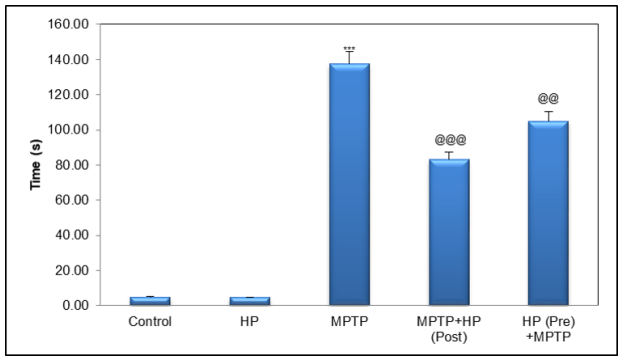

Figure 3: Effect of Hypericum perforatum on behavioural studies using Catalepsy test score.Results are measured as Mean ± SEM(n=6)

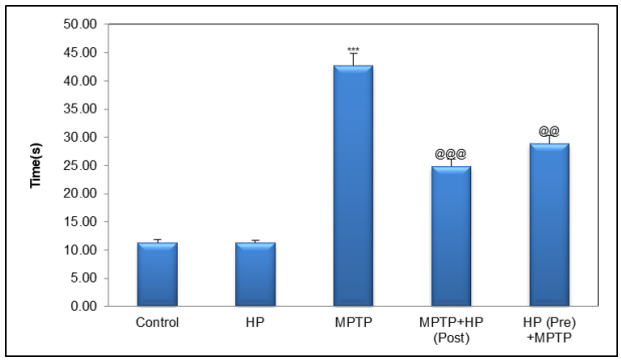

Figure 4: Effect of Hypericum perforatum on behavioural studies using Pole test scores. Results are measured as Mean ± SEM (n=6)

RESULTS AND DISCUSSION

The novel therapeutic strategies able to modify the course of neurodegenerative disorders are currently one of the major goals for the researchers of this area. Developing novel pharmaceutical compounds having antioxidant properties will remarkably increase our understanding of their functions besides radical species in biological systems. The forced swim test (FST) is more appropriate for animal models with bilateral lesions and with partial dopamine diminution. Such animal models are appropriate for drug discovery studies, and

thus, FST can serve as a valuable tool for assessing motor behavioural aberration in neuroprotective studies (Chonpathompikunlert *et al.*, 2018).The FST was used for evaluating depressive-like behavior, as described previously (Chen *et al.*, 2015). In the present study, HP alone treated mice showed non-significant results when compared with control mice. Rodents administered with MPTP showed a significant increase in swim test scores when compared to the control group. The swim test scores were found decreased in the pre and post *Hypericum perforatum* extract (HPE) treated group when compared with MPTP administered mice (Figure 1). ***p<0.001 statistically significant from the control group; $^{@@}$p<0.01, $^{@@@}$p<0.001 statistically significant as compared with MPTP treated group.

Similar to the FST, in the tail suspension test, mice were kept in an inescapable but mild stressful situation. The lack of associated escape behavior is measured as immobility (Ramalhete *et al.*, 2016). In the tail suspension test, MPTP administered mice showed a significant increase in tail suspension test scores when compared to the control group. Reduced tail suspension scores were found in HPE pre and post-treated mice when compared with the MPTP group of mice (Figure 2). ***p<0.001 statistically significant from the control group; $^{@@}$p<0.01, $^{@@@}$p<0.001 statistically significant as compared with MPTP treated group.

This immobility might be associated with a difference in stress-induced behavioral depression and can be linked with the psychological construct of frame-up seen in clinical depression (Kiasalari *et al.*, 2016). As a pointer of the antidepressant effect, it was observed that the immobility time of animals in the tail-suspension tests was shorter, i.e. the effect of the animals was greater in HPE treated mice.

The results of this administration of H. perforatum plant extract using forced swim test model display antidepressant effects several times lower than those in post-treatment experiments. Hesperidin, an active component present in HPE, was observed to be effective in reducing cognitive and depressive deficits by controlling neurotransmitter systems (Zirak *et al.*, 2019). It has been shown recently that a flavonoid fraction obtained from a crude extract of *Hypericum perforatum* was remarkably active in the forced swimming test (Kordjazy *et al.*, 2016). Future trials are required to further characterize the mechanism of the antidepressant action of H. perforatum, particularly of single compounds of the extract, as the only natural drug with antidepressant action. The tail suspension tests are acute trials of antidepressant effect that depends on behavioral despair or immobility to detect depressive-like behavior of mice.

The pole test is generally used for measuring basal ganglia related mobility diseases (Pałucha-Poniewiera *et al.*, 2017). MPTP-administered mice are proposed as a useful tool for measuring the capacity of pharmacological agents to hinder recognition deficits in PD. Rodents administered with MPTP showed a significant increase in pole test scores when compared to the control group. Decreased pole test scores were found in the post-test group when compared with MPTP administered mice (Figure 3). ***p<0.001 statistically significant from the control group; $^{@@}$p<0.01, $^{@@@}$p<0.001 statistically significant as compared with MPTP treated group.

Catalepsy (immobility reflex, tonic immobility, and animal hypnosis) is considered by muscular rigidity resulting in prolonged immobility and an inability to correct an externally imposed awkward posture. Excessive catalepsy-like dyskinesia in human is a pathological symptom happening in mood disorders (e.g. depression), schizophrenia, and Parkinson's' disease (Poleszak *et al.*, 2019).Cataleptic behavior in the PD mice has been hired as a standard model of bradykinesia and rigidity in human Parkinson's disease. The characteristic catalepsy test involves keeping mice into an unusual posture and recording the time taken to come back to the normal posture. Catalepsy, the mobility impairment, is an extrapyramidal dysfunction called a prominent motor symptom of PD, which is associated with striatal dopamine reduction (Vajdi-Hokmabad *et al.*, 2017). The MPTP administered rats showed a significant increase in catalepsy test scores when compared to the control group. The decreased catalepsy scores were found both in the HPE pre and post-treated group in comparison with MPTP administered mice (Figure 4). ***p<0.001 statistically significant from the control group; $^{@@}$p<0.01, $^{@@@}$p<0.001 statistically significant as compared with MPTP treated group.

The results of this pretreatment study on mice after H. perforatum plant extract therapy proved antidepressant effects several times lower than those in pretreatment experiments. Hesperidin, an active component present in HPE, was observed to be effective in reducing depressive and cognitive deficits in mice via modulating neurotransmitter systems (German-Ponciano and Rosas-Sánchez, 2018). It has been proved recently that a flavonoid content taken from *Hypericum perforatum* extract was remarkably active in the FST (Ben-Eliezer and Yechiam, 2016). Future trials are required to further

characterize the mechanism of the antidepressant action of H. perforatum.

CONCLUSION

Hypericum perforatum extract ameliorated depressive-like *behavior* better in post treatment as compared with pretreatment group in MPTP model of *Parkinson's* disease. It can be said that methanolic extract of *Hypericum perforatum* can be a therapeutic agent for Parkinson's treatment, however further clinical studies are required.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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