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Efficacy of *Hypericum perforatum* extract on ultrastructural changes in brain and oxidative damage in Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is a serious chronic and progressive neurological disorder. *Hypericum perforatum* (*H. perforatum*) is a plant generally used as an antidepressant and therapy for many neurodegenerative disorders. The present study evaluates the action of *Hypericum perforatum* extract on MPTP induced oxidative stress and ultrastructural changes in the Parkinson disease model. For this research study, Parkinson disease in mice were induced using MPTP and Hypericum perforatum methanolic extract were given in the pre-treatment and post-treatment condition. After the treatment, the oxidative stress was measured using TBARS levels and the activities of GSH, GPx, CAT and SOD in brain tissues. The ultrastructural changes in brain mitochondria caused by MPTP toxicity was studied after *Hypericum perforatum* extract therapy using TEM images. The present study showed that HPE extract is able to balance the oxidative stress and antioxidants in the brain tissue of MPTP administered rats. The damaged nuclei in the brain recovered to the normal condition due to this extract therapy. Finally, to conclude, *Hypericum perforatum* extract post-treatment offers potential brain protection against MPTP-induced Parkinson's disease than pre-treatment.

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INTRODUCTION

Apoptosis of a minor group of brain cells that control body movements leads to Parkinson's disease. Oxidative stress plays a significant role in the sporadic forms of Parkinson's disease. Production of reactive oxygen species (ROS) happens during intracellular pathways, and they trigger oxidative stress in the PD model. MPP^+ displays a high attraction for the dopamine transporter (DAT) and is transformed into Dopaminergic (DA) neurons, where it injures respiration by hindering mitochondrial complex-1, which results in the formation of increased ROS. ROS arouses cell death via oxidatively injuring molecules such as hydroxyl and superoxide radicals and leads to lipid and protein peroxidation. Then, the affected DA neurons can degenerate either by apoptosis or by necrosis (Zheng *et al.*, 2017).

MPTP (1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine) selectively injures dopaminergic neurons of

substantia nigra leading to the reduction of their endings in the striatum and the reduction in the number of neurons in the pars compacta of substantia nigra. Mitochondrial dysfunction is one of the most significant hallmarks of PD pathogenesis. If mitochondria are affected by the disease, the cell stops functioning well. It has long been recognised that mitochondrial dysfunction is critically included in the demise of dopaminergic neurons in PD. Eventually, in TEM photographs, small electron-dense deposits were found in mitochondria of PD subjects, which are exclusively regenerated in dopamine-treated cells (Zhang *et al.*, 2016).

Indigenous antioxidants can be used for the prevention of deleterious consequence of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in herbs, spices, and medicinal plants (Tysnes and Storstein, 2017). Though there are various synthetic antioxidants currently in use, there is growing evidence of consumer preference for natural antioxidants due to their lower toxicity. For the effective management of PD, the need for alternative medicine, without adverse effects, and with neuroprotective effects progresses (Lan *et al.*, 2016).

Hypericum perforatum is a significant medicinal herb that can be used for treating mental illnesses (Oliveira *et al.*, 2016). It has been used as a medicinal plant for centuries for treating external and internal disorders. H. perforatum can be used for treating minor burns, skin inflammation, wounds, and nerve pain (Oliveira *et al.*, 2016). Internally, it is used for the treatment of anxiety and depression, competing for status as a standard antidepressant therapy (Herraiz and Guillén, 2018). The present study was done to analyse the effects of *Hypericum perforatum* on antioxidant changes in MPTP induced Parkinson's disease.

MATERIALS AND METHODS

Animals

The C57BL/6 male mice, 3months old, 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 research on animals" (CPCSEA guidelines). The animals were kept in polycarbonate cages in the standard day-night cycle and the temperature kept at 22 ± 2^0C. The rats 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 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 (Lan *et al.*, 2016).

Plant Material and Preparation

Hypericum perforatum plant extract was purchased from JK medicinal plants introduction centre (JKMPIC), Srinagar, Jammu and Kashmir with authentication (no: JKMPIC-(K) R&D 20119). The air-dried sample was powdered using an auto-mix blender and the methanolic extract prepared using Soxhlet apparatus and concentrated using a rotary evaporator at 40°C and stored in a cool place. The residue was evaporated to dryness and was dissolved in water for further studies.

Experimental Grouping

The animals were grouped into 5 groups, each containing 6 animals.

Group 1- Normal 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 received MPTP (30mg/kg, i.p) for 5 consecutive days as a single dose.

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

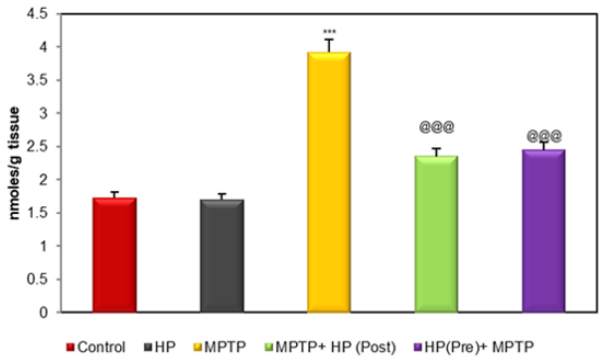
Group 5- Mice pretreated orally with HPE (300mg/kg b.w) and then injected with MPTP (30mg/kg, i.p) for 5 consecutive days.

The brains were excised immediately; the striatal region was identified using the stereotaxic of a mice brain.

The fresh brain was serially sectioned and the striatum was separated. The tissue was homogenised in an ice-cold solution of 0.1% M PBS and used for further analysis (Ko *et al.*, 2018).

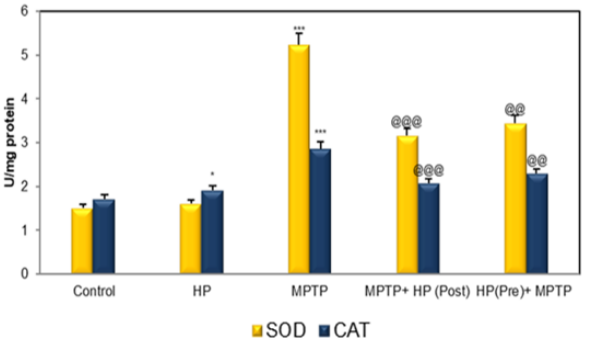
Estimation of Lipid Peroxidation Products

Lipid peroxidation products were measured by the method of Rems *et al.* (2019). To the test, samples added 5 ml of TBA reagent and then colour changes to pink, which was read at 532 nm using a visible spectrophotometer. The Thiobarbituric Acid Reactive Substance (TBARS) was formed as a product that was measured and expressed as n moles of MDA formed /min/mg protein.



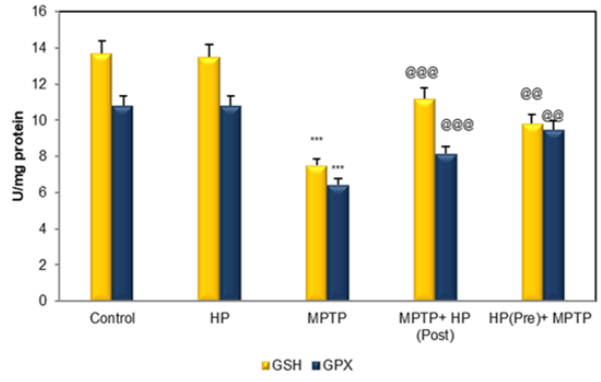
**p<0.01, ***p<0.05 statistically significant in comparison to control group; @p<0.05, @@p<0.01, @@@p<0.01 statistically significant in comparison to MPTP injected group

Figure 1: Effect of *Hypericum perforatum* on TBARs levels in MPTP induced Parkinson disease



**p<0.01, ***p<0.05 statistically significant in comparison to control group; @p<0.05, @@p<0.01, @@@p<0.01 statistically significant in comparison to MPTP injected group

Figure 2: Effect of *Hypericum perforatum* on SOD and CAT activities in MPTP induced Parkinson disease



***p<0.001 statistically significant in comparison to control group; @@@p<0.001 statistically significant in comparison to MPTP injected group

Figure 3: Effect of *Hypericum perforatum* on GSH and Gpx activities in MPTP induced Parkinson disease

Assay of Superoxide Dismutase

This enzyme activity was measured according to the procedure of Zhang *et al.* (2016). The chemical reaction such as oxidation of adrenaline to adrenochrome was taken place in an incubation mixture of (3 ml) having 0.1 mM EDTA, 0.05 M Na_2CO_3, sample (100 μl) and 3×10^{-4} M adrenaline (100 μl). The auto-oxidation inhibition by SOD in the samples was observed spectrophotometrically (480 nm$\times$26ºC$\times$4 min) and the enzyme activity in brain tissues was expressed as Units/min/mg of tissue protein.

Assay of Catalase

Catalase activity was measured according to the procedure of Hadwan (2018). About 20 μl of the tissue homogenate was added in an incubation mixture containing buffer 50 μl, 900 μl of an H_2O_2 substrate and distilled water 30 μl. Hydrogen peroxide degradation was measured spectrophotometrically for 3 minutes at 230 nm, 37ºC. One unit of CAT is measured as the μmol H_2O_2/min/mg protein under specified conditions.

Assay of Glutathione Peroxidase

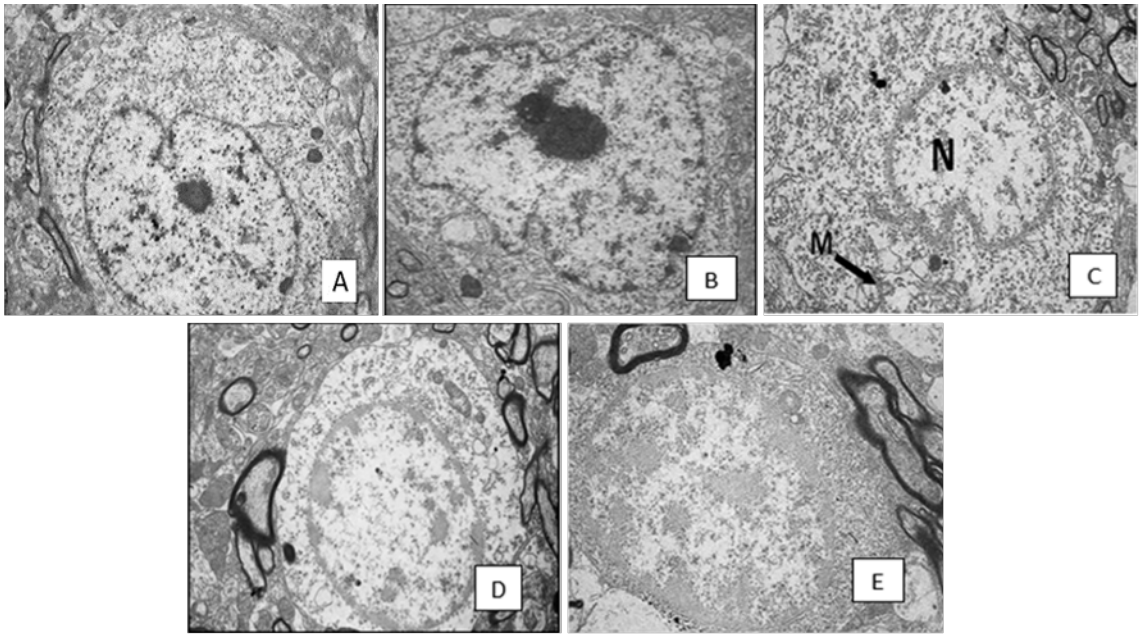
The activity of the GPx enzyme in brain tissue was measured by the method of Li *et al.* (2018). Glutathione peroxidase activity was analysed depending on the fact that GPX catalyzed the oxidation of glutathione by cumene hydroperoxide. The oxidized form of glutathione converted to the reduced glutathione form with the association of NADPH oxidation to $NADP^+$. For evaluating GPX activity in samples (10 μL) was added with GPX assaying reagent (500 μL) and cumene (20μl). The absorbance was noted at 340 nm and the GPX enzyme activity were measured in U/mg protein.

Estimation of Reduced Glutathione

GSH levels in tissue were estimated by following Morales-Kastresana *et al.* (2019) procedure. Momentarily, 1.5 mL of precipitating reagent and 0.9 mL distilled water were added to 0.1 mL of the sample. It was mixed and incubated at 37°C for 5 min. The mixture was centrifuged (4000 rpm$\times$ 4°C$\times$15 min). The phosphate solution (4.0 mL) and DTNB (0.5 mL) was added to 1.0 mL supernatant. The production of the yellow colour complex was read spectrophotometer at 412 nm. The concentration of GSH was measured as μmol of U/mg protein.

Transmission Electron Microscopy (TEM)

The brain tissue sections was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and washed with 7% sucrose buffer; the specimens were then post-fixed in 1% OsO_4 for 2 h at 48°C and



A – Control, B – HP alone treated, C – MPTP alone, D – MPTP + HP (Post), E – HP (Pre) + MPTP. (marking M-Mitochondria; N – Nucleus)

Figure 4: Effect of *Hypericum perforatum* on ultrastructural changes by Transmission electron microscopy in MPTP induced Parkinson disease

washed 3 times in buffer. Then the specimens were dehydrated in acetone, the tissues passed through propylene oxide and finally embedded in epoxy resin. Ultrathin sections (60 nm) were placed on copper grids and added uranyl acetate and lead citrate stain. The tissue section was observed using Transmission Electron Microscope Zeiss EM109 at 100 kV (Costa *et al.*, 2019).

Statistical Analysis

The results expressed as Mean ± Standard Error Mean (SEM) (n=6). The statistical significance was observed by one-way ANOVA using SPSS version 21.0 and the statistical comparisons between the groups were measured by Duncan's test. A P value of less than 0.05 was considered to be significant between groups.

RESULTS AND DISCUSSION

For Parkinson's disease patients, L-DOPA therapy was given which has serious side effects after a long duration of treatment (Wei *et al.*, 2018). The antioxidants SOD, CAT and GSH effectively inhibit oxygen free radicals and lipid peroxides formation, which may be used to measure the percentage of oxidative stress. Natural antioxidants are enhanced in herb medicines may have neuroprotective action in PD by increasing the activities of GSH-Px or SOD (Wei *et al.*, 2018). The effect of *Hypericum perforatum* on striatal TBARS levels has been shown in Figure 1. The effect of *Hypericum perforatum* on antioxidant enzyme activities has been given in Figure 2 and Figure 3. Non-significant changes were found in HP alone treated rats for all the parameters except for CAT, which showed an increase in antioxidant status when compared to control rats. The present study found that GSH and Gpx activity was reduced, whereas SOD, CAT as well as TBARS level were hiked in MPTP rats in comparison to control rats. Pre and post HPE treatment significantly increased GSH and Gpx activity whereas significantly reduced the SOD, CAT activities and TBARs level in MPTP administered rats.

These present reports indicate that HPE possesses an antioxidant property due to the presence of flavonoids and phenolic compounds that decrease the total peroxide level and oxidative stress index and inhibits oxidative stress, which is consistent with a previous study (Loeffler *et al.*, 2017). Another research study insists that HPE exert an antioxidant effect by reducing MDA level reduction while increasing GPx activity and the % inhibition of O_2 (Miletić *et al.*, 2018). It has been shown that MAO-B inhibition was apparent with *H. perforatum* extract fractions having higher concentrations of flavonoids. Possibly due to inhibition of MAO-B, conversion of MPTP to MPP+ is decreased, resulting in decreased lipid peroxidation (de Farias *et al.*, 2017).

Cao *et al.* (2017) showed that a free radical scavenging effect against superoxide anions could be due to the presence of hypericin. *H. perforatum* extracts are already known for their anti-lipid peroxidative property *in vivo* (Zirak *et al.*, 2019). The *H. perforatum* extract has various phenolic compounds, including flavonoids and phenolic acids, signifying that they could have significant anti-inflammatory and antioxidant properties (Tanideh *et al.*, 2020).

In the present report, HPE increased the antioxidant enzyme activities such as GSH, and Gpx, whereas decreased SOD, CAT activities and TBARs level in MPTP administered animals.

The pathological changes in neuronal tissue of Parkinson's disease (PD) are a loss of dopaminergic (DA) neurons in the mesencephalon and showed the presence of Lewy bodies (Mahoney-Sánchez *et al.*, 2021). TEM image (Figure 4) show that control rats (Figure 4A) have normal striatal neuron with normal nuclei. HP treated rats (Figure 4B) showed normal nuclei with dispersed chromatin with the normal nuclear membrane.

The present study showed that MPTP administered rats (Figure 4C) have smaller nuclei with aggregated high-density chromatin gathered near the nuclear membrane and the starting phase of nuclear membrane degeneration. It also showed swollen and loosely arranged Golgi apparatus with less number of ribosomes. HP post-treated (Figure 4D) and HP pre-treated (Figure 4E) MPTP rats showed normal Golgi apparatus with moderate ribosomes and normal size nuclei without nuclear membrane damage, as well as moderate chromatin aggregation.

More number of dopaminergic neurons and apoptosis was seen in the substantia niagra region in the MPTP group, and treatment with HPE reduced the toxicity of MPTP in brain tissue (Ko *et al.*, 2018). All these findings support that HPE offers neuroprotection against MPTP-induced Parkinson's disease model.

CONCLUSION

The present study showed that *H. perforatum* extract is able to reduce oxidative stress and improves the ultrastructural changes in brain tissue of the Parkinson's model. Thus, this scientific justification provides strong evidence for this plant as the best medicinal plant for the prevention or treatment of Parkinson's disease.

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The authors declare that they have no funding support for this study.

Conflict of Interest

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

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