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RESEARCH ARTICLE

Antiparkinsonian Activity of Hydroalcoholic Extract of *Operculina turpethum* Roots in Reserpine Induced Parkinsonism in Wistar Rats

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Abstract

Objective: The aim of this study was to examine the effect of *Operculina turpethum* (Linn.) root extract in attenuating reserpine induced Parkinson's disease to quench the free radicals produced as a result of the increased oxidative stress in Parkinson disease. **Methods:** Parkinson's disease is induced by administration of reserpine (0.1 mg/kg/day, i.p.) in albino rats for 21 days. Simultaneously the rats were pretreated with two doses of hydroalcoholic extract of *Operculina turpethum* (200mg/kg and 400mg/kg p.o.) daily once for the 21 days. The anti-Parkinson's effect of the extract was evaluated by assessing various behavioral (motor and non-motor) and biochemical (lipid peroxidation, creatinine, acetylcholine esterase and total protein) parameters as well as histological examination. **Findings:** Reserpine significantly causes tremor, rigidity, akinesia which were reversed by daily administration of *Operculina turpethum* root extract that may be attributed to a reduction in the acetylcholine, total protein, and creatine due to multifunctional activity of various phytochemicals present in the *Operculina turpethum* extract when compared to L-DOPA/C-DOPA group. There was a significant histological improvement in the neuronal degeneration in brain tissue on treatment with *Operculina turpethum* root extract. The results indicated the protective effect of *Operculina turpethum* extract against Parkinson's disease. **Novelty:** Dopamine replacement and levodopa, two prevalent medications for PD, only exhibit some effects of limited symptomatic relief but cause many severe adverse effects, such as hallucination and involuntary movement. The hydroalcoholic extract of *Operculina turpethum* roots can be favourable for the development of disease-modifying drugs for Parkinson's disease.

Keywords: *Operculina turpethum*; Parkinson's Disease; Reserpine; Lewy bodies; Neuroprotective

1 Introduction

Parkinson's disease (PD) exerts a significant impact on society. It is a prevalent condition, affecting around 7.1 million people worldwide in 2021, and its incidence and prevalence have notably increased over the past two decades, a trend whose underlying reasons remain incompletely understood[(1)]. This surge in cases will impose a growing economic and social burden on aging populations in industrialized societies[(2)]. PD has been characterized by its classical motor symptoms associated with the existence of Lewy bodies and the damage of dopaminergic neurons in the Substantia Nigra (SN)[(1)]. PD is a progressive neurological disorder with a multitude of motor and non-motor features, affecting individuals to varying degrees[(3)]. It typically manifests in older individuals but can also occur in younger patients, making it the second most common neurodegenerative disease[(4)]. The Movement Disorder Society recommends using a modified version of the banded Parkinson Disease Rating Scale (UPDRS) to assess disease progression, encompassing aspects of cognition, mood, daily living activities, motor examination, and complications encountered during motor examinations, with responses scaled from normal to severe[(5)]. To date, the pharmacological treatments used in PD are exclusively symptomatic. For this reason, in recent years, the research has been directed towards the discovery and study of new natural molecules to develop potential neuroprotective therapies against PD.

Operculina turpethum (Linn.) (*O. turpethum*), a plant belonging to the morning glory family, also known as turpeth, is a perennial medicinal plant belonging to Convolvulaceae family. This plant kingdom is known to be a rich source of bioactive compounds with potential in managing various challenging diseases[(6)]. Reports suggest that *O. turpethum* contains bioactive constituents such as saponins, flavonoids, steroids, triterpenes, and other substances that may possess neuroprotective or anti-Parkinsonian activities. Although the plant in question contains high concentrations of these bioactive compounds, there is currently no literature evidence regarding its use in anti-Parkinsonian therapy[(7)]. In this context the present study has been conducted to evaluate the potential therapeutic role of natural *O. turpethum* extract in PD.

2 Materials and methods

2.1 Collection and authentication of *O. turpethum* roots

The roots of *O. turpethum* was collected from Bangalore, Karnataka, India. The herb was recognized by taxonomist at the Central Ayurveda Research Institute, Thalaghattapura post, Bengaluru-560109, as *O. turpethum* Linn family Convolvulaceae. With authentication letter no: SMPU/CARI/BNG/2022-23/2583 Dated 24/02/2023.

2.2 Extraction of plant material

The hydroalcoholic extract of *O. turpethum* roots (HEOT) was collected from Green Chem, Bengaluru, Karnataka, India. The extract was obtained in the form of a dry powder. Hydroalcoholic extracts were prepared using Soxhlet's extractor. The extracts were filtered and dried. The extracts were administered in doses of 200 and 400 mg/kg (p.o.). Control group was given only vehicle in equivalent volume of plant extract.

2.3 Phytochemical screening[(8)]

Chemical identification test for detection of steroids, glycosides, flavonoids, alkaloids, and triterpenoids were performed as per the standard procedure.

2.4 Experimental animals

All experiments were performed on Sprague Dawley rats of either sex weighing 200 $\pm$ 50g. The animals were procured from the in-house animal facility of Krupanidhi College of Pharmacy, Bangalore. Animals were housed in group of 6 per cages, maintained at 23$\pm$2 °C; 55$\pm$5% humidity in a natural light and dark cycle, with free access to food and water. The experiments were performed during the light cycle in awake, freely moving animals that were adjusted to laboratory conditions before proceeding with the experiments. All animal procedures were approved by the ethical committee at our institution (Approval no: KCP/IAEC/PCOL/112/2022) and performed in compliance with institutional guidelines for the care handling of experimental animals and as per CPCSEA.

2.5 Experimental Design - Reserpine induced Parkinson's disease[(9)]

The animals were divided into five groups of six rats. HEOT was administered at a dose of 200 and 400 mg/kg. L-dopa + carbidopa was administered at a dose of 9 +1.8 mg/kg. Reserpine was given to all groups at the dose of 0.1 mg/kg i.p. once in

a day for 21 days consecutively. Pre-treatment with HEOT and L-DOPA/C-DOPA was given simultaneously for 21 days and observations were made after 24 hours of the last treatment of reserpine. The time interval between administration of extract and reserpine was 60 mins. All the groups have undergone behavioral biochemical and histopathological tests. The drug was administered orally except group 1 and 2. The extract/drug were given in volumes of 10 mL/kg [Table 1]. At the end of the study the rats were sacrificed by instant decapitation.

Table 1. Experimental design

Group	Treatment	Dose mg/kg
1	Negative control (Vehicle)	10mL/kg p.o.
2	Positive control (Resrpine)	0.1 mg/kg i.p.
3	Low dose of HEOT (LHEOT)	200 mg/kg p.o.
4	High dose of HEOT (HHEOT)	400 mg/kg p.o.
5	Standard drug - L-Dopa + Cardbidopa (L-DOPA/C-DOPA)	9 + 1.8 mg/kg p.o.

2.6 Behavioural assessment

2.6.1 Catalepsy test

Cataleptic behaviour was evaluated by placing the animal's front paws on a horizontal bar located 9 cm above the bench surface. The duration of catalepsy, characterized by a motionless posture with both front paws on the bar, was recorded, with a maximum limit of 180 seconds. Each animal underwent three trials on each observation day, and the results were recorded [10].

2.6.2 Open field test

Rats were gently placed in the central area and allowed 5 minutes of free exploration. A mounted video camera recorded their Behaviour for later analysis. Key behavioural parameters, including the time it took for the rats to move all four paws, rearing activity, Number of centre square entries, Number of line crossings and Centre square duration were measured [11].

2.6.3 Elevated plus maze test

On the first and second day of testing, individual rats from each experimental group were placed on the central platform, initially facing the open arm, permitted to freely explore the maze for five minutes. A digital camera was used to record behavioural observations [12].

2.6.4 Rotarod test

Rats were placed on the treadmill and the Rotarod accelerated from 0 to 40 rpm over 60 seconds. Latency to drop was recorded upon falling [13].

2.6.5 Actophotometer

Actophotometer, containing photoelectric cells connected to a counter was used to measure locomotor activity. Over the course of five minutes, a count was recorded whenever the reserpine-treated animal blocked the light beam from reaching the photocell [14].

2.6.6 Forced swim

Rats were placed in water container (22°C - 25°C) for 30 minutes. Their swimming ability and limb usage were observed. Rodents typically avoid water and tend to float, particularly when stressed. However, the initial dopamine loss only temporarily impacted swimming performance, making this test inadequate for assessing basal ganglia impairment and immobility time is recorded. [15]

2.6.7 Histopathological analysis

After euthanizing the animals, the brains were quickly removed and washed with ice cold saline and were stored in 10% formalin fixative solution. Histopathological assessment of brain was done at Koushik Laboratory and Clinic in Bengaluru [16].

2.7 *In-vitro* Analysis

2.7.1 Tissue homogenate

Animals were sacrificed by spinal dislocation immediately after behavioural assessments. The complete brain was removed and 10% (w / v-1) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). Homogenate was centrifuged for 20 minutes at 15000 rpm and the supernatant was used for biochemical assay[16].

2.7.2 Lipid peroxidation (LPO)

The amount of malondialdehyde (MDA) in the tissue homogenate was measured by reaction with (TBA) thiobarbituric acid 535 nm using Shimadzu Spectrophotometer. The values were calculated using molar extinction coefficient of chromophore and expressed as n moles formed per mg of protein in the tissue. The findings were presented as MDA/l mmol[17].

2.7.3 Acetylcholinesterase activity

The colorimetric approach was applied to tissue homogenate samples with minor modifications to evaluate the catalytic activity of AChE. Using a UV-VIS Spectrophotometer, the change in absorbance was monitored for two minutes at one-minute intervals at 412 nm. Acetylthiocholine iodide micromoles hydrolysed per minute per milligram of protein represented the acetylcholinesterase activity[18].

2.7.4 Estimation of total brain protein

The total brain protein was estimated using an Erba kit through an autoanalyzer[19].

3 Results and Discussion

PD is a disorder of the central nervous system, involving primarily a degeneration of certain nerve cells in deep parts of the brain called the basal ganglia, and in particular a loss of nerve cells (or neurons) in a part of the brainstem called the substantia nigra. These cells make the neurochemical messenger dopamine, which is partly responsible for starting a circuit of messages that coordinate normal movement. Normally used anti-Parkinson's show severe adverse effects when used for long term. Herbal extracts have raised much attention for their important anti-inflammatory and antioxidant properties, but also for their ability to modulate protein misfolding. Therefore, this study was conducted to evaluate the effect of *O. turpethum* extract in PD.

In present study, the preliminary phytochemical screening of the *O. turpethum* roots has ascertained the presence of glycosides, steroids, flavonoids and alkaloids. The phytochemical analysis conducted by Karmakar et al. showed the presence of reducing sugar, phenolic compounds, tannins, flavonoids, glycosides, alkaloids, in the extract of *O. turpethum* roots which is similar to the present study[20].

When rats were given low doses of reserpine repeatedly, motor symptoms gradually started showing up that resembles the pathogenesis of PD similar to PD animal models induced by some other researches[15]. An increased degree of oxidative stress is present along with these motor deficits, and this has been proposed as the likely pathophysiological role in PD (PD). To highlight pathological feature of PD, we used a chronic regimen of low-dose reserpine (0.1 mg/kg) over a period of 21 days in our current study.

Motor functions (rotarod, catalepsy, open - field), non-motor functions such as anxiety (elevated plus maze) and depression (FST) were evaluated. Regarding motor learning, the reserpine-treated group exhibited significantly reduced time spent on the rotating rod in comparison to the normal control group. Giri et al. observed *Nardostachys jatamansi* (30, 100 mg/kg), *Mucuna pruriens* (30, 100 mg/kg) and 1:1 combination mixture (30, 100 mg/kg) significantly inhibited haloperidol induced catalepsy. As compared with the control group 1:1 mixture (30, 100 mg/kg) showed extremely significant changes. The effect was observed due to the presence of levodopa, jatamansone and coumarinis as active chemical constituents present in the extracts along with supportive constituent and their antioxidant properties[21].

Reserpine treated rats when subjected for motor function; we observed a progressive development of motor impairment. The negative responses in these motor test indicates the main motor PD symptoms, such as hypokinesia in the open field, slowness / decline in initiation of movements during the catalepsy test and losing its grip strength when these rats were subjected to rotarod test. When compared to the normal control group, the group treated with reserpine displayed increased cataleptic behaviour. As opposed to the diseased control group, the rats receiving HEOT treatment demonstrated significant decrease in cataleptic activity on day 14 ($p<0.001$) and on day 21 ($p<0.001$) (Figure 1). The reserpine-treated group exhibited significantly reduced time spent on the rotating rod in comparison to the normal control group. In contrast, the high dose of HEOT treatment produced relatively effective results when compared to the low dose of HEOT treated groups. The standard drug

L-Dopa/Carbidopa demonstrated a relatively positive impact [22]. Daidzein (50 and 100 mg/kg, p.o.) and the combination of L-dopa and carbidopa with daidzein a plant derivative produced a significant reduction in these symptoms in rats [16].

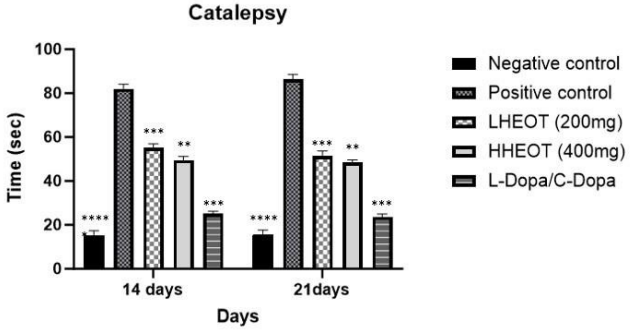

Fig 1. Graphical representation of the effect of HEOT in reserpine prompted catalepsy

On the 14th day of repeated reserpine treatment when tested in an open field, the animals displayed reduced distance travelled and less time spent in the inner zone, while total distance and average speed remained unaffected. On the 21st day, significant treatment effects were observed in total distance travelled, but not in average speed, time in the inner zone, and distance travelled in the inner zone. The decrease in total distance travelled on the 21st day suggests a potential change in motor behaviour due to reserpine administration (Figure 2). In contrast, the treatment group showed increased distance travelled in the inner zone and total distance travelled at an average speed compared to the disease (reserpine-induced Parkinson's) control group. HEOT treatment yielded similar results to L-dopa/Carbidopa on corresponding days that resembled with finding of some researchers [23].

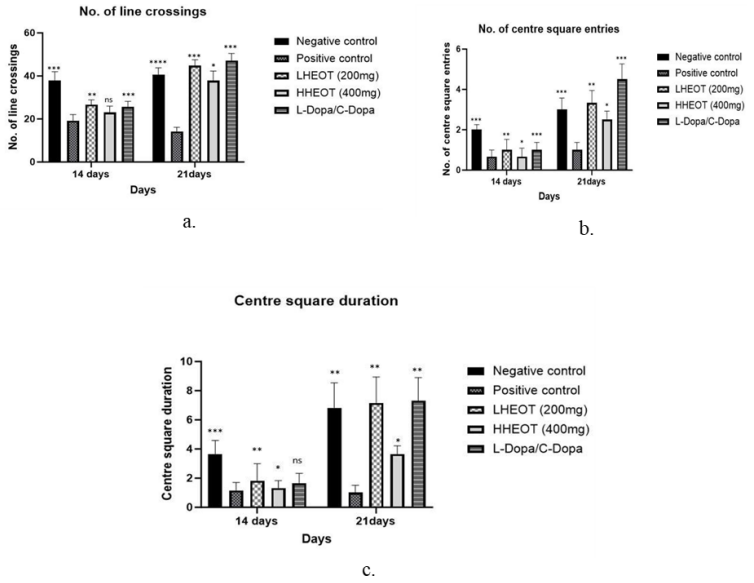

Fig 2. Graphical representation of effect of HEOT on open field test. a. Number of line crossing; b. Number of centre square entries; c. Centre Square duration

PD is elementally a disturbance in motor; patients show similar debilitated nonmotor feature (anxiety, depression) that may become visible much earlier or concomitantly to motor signs. In the anxiety test model, when comparing to the normal control group, the reserpine- treated group showed a significant increase in both the first and second transfer latency on day 21, relative to the initial transfer latency (ITL) measured on day 14. In comparison to the untreated reserpine induced PD group, HEOT

therapy significantly reduced the reserpine-induced increase in first transfer latency and second transfer latency on day 21 ($p < 0.0001$), indicating that HEOT had a significant antianxiety effect which was also proved by Swami et al. in the study of *Ipomoea turpethum* extract loaded polymeric nanoparticles in Wistar rats [24].

Depression is repeatedly observed in PD. A depressive behaviour in reserpine induced rats was tested using forced swimming test (FST). The reserpine-treated group exhibited higher levels of immobility in comparison to the control group (Figure 3). In contrast, high dose HEOT treatment produced relatively less pronounced results compared to the low dose of HEOT *O. turpethum* treated groups. HEOT treated group was comparable with the standard drug L-Dopa/Carbidopa drug therapy. Herbal extracts have shown antidepressant activity which is in agreement with the present study [25].

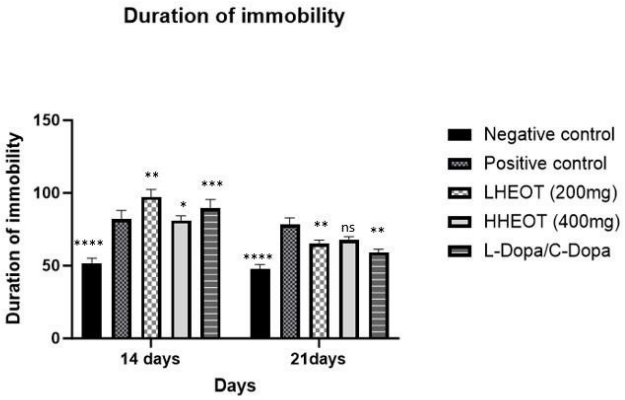

Fig 3. Graphical representation of the effect of HEOT on forced swim test (Duration of immobility)

The activeness was measured by using actophotometer, and results showed that the group receiving reserpine had less locomotor activity than the normal control group. Comparing the high dosage HEOT treatment groups to the low dose HEOT treated groups, the effects were comparatively less noticeable (Figure 4). L-Dopa and Carbidopa, the conventional medication, had a comparatively beneficial effect and was comparable with studies done elsewhere, that validated the beneficial effect of *Psidium guajava* and *Madhuca indica* extracts in the treatment of PD.

The motor coordination in the rats was measured by rotarod test. The rats treated with hydroalcoholic extract of *O. turpethum* roots on rotarod test showed effective motor coordination as compared to positive control group. The reference standard L-Dopa and Carbidopa, the conventional medication, had comparatively beneficial effect (Figure 5).

Goel and Chaudhary have reported that daidzein a plant-derived diphenolic compound of the phytoestrogens class was also able to increase the locomotor activity and grip strength in rats. Reserpine induced motor defect was significantly reversed by daidzein. Amelioration of symptoms of reserpine by daidzein demonstrates anti-Parkinson's activity [16]. The neuroprotective activity of the daidzein and *O. turpethum* extract may be due to its antioxidant property, which reinforces anti-Parkinsonian activity of daidzein.

The well-known useful medication for PD, such as levodopa and dopamine replacement have exhibited limited symptomatic relief and showed many adverse effects that are very severe in nature. In the absence of disease modifiers, herbal medication is perfectly the alternative source that has been gradually useful in the various disease including PD due to multifunctionality of phytoconstituents and their remarkable efficacy with lesser adverse effects. Numerous herbal medications have been used in the past to treat neurodegenerative diseases and their phytochemicals might be helpful in disease modifying or to slow down the progression of PD [21].

3.1 Lipid peroxidation test

The deterioration of certain cellular pathways has led to oxidative distress and malfunctioned protein overlap, transmit and degradation, finally the Lewy's bodies aggregates and causes cell death. So, PD is primarily related to production of oxidative stress that has led to gathering of defective proteins, thus resulting in production of toxic reactive oxygen and nitrogen species that has led to cell damage and cell death. The cellular stress and neurodegeneration in PD are due to oxidative imbalance and reserpine induced animal model PD is aimed to reproduce the same as it causes cellular stress and molecular damage. Preclinical therapeutic strategies aim to target the above-mentioned pathway to slowdown the progression of PD. The impact of HEOT on reserpine-induced PD on malondialdehyde (MDA) was investigated. When compared to the control group receiving

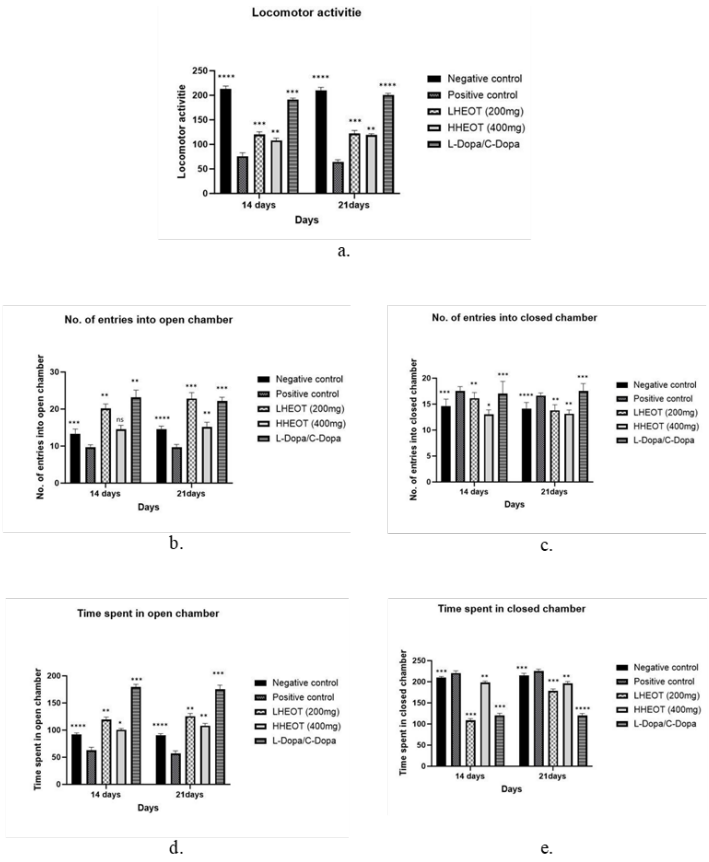

Fig 4. Graphical representation of the effect of HEOT on actophotometer test. a. Locomotor acitivty; b. Number of entries in to open chamber; c. Number of entries in to closed chamber; d. Time spent in open chamber; e. Time spent in closed chamber

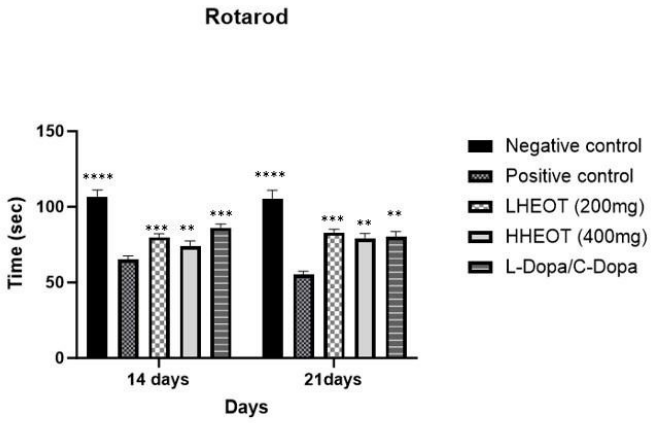

Fig 5. Graphical representation of effect of HEOT roots on rotarod test (Time)

a vehicle, the administration of reserpine led to notable alterations in various biochemical parameters especially MDA. Notably, the treatment with HEOT resulted in a substantial and statistical significant decrease in the level of MDA with a p-value of less than 0.001 (Figure 6) and was comparable with other study [16].

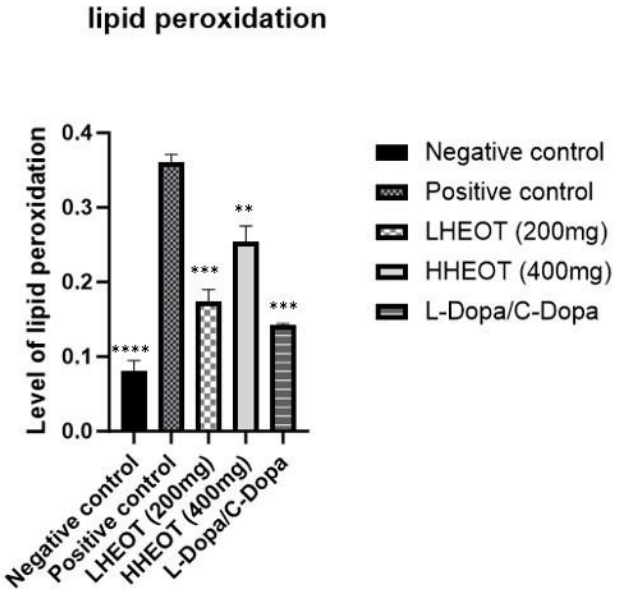

Fig 6. Graphical representation of the effect of HEOT on level of lipid peroxidation

3.2 Acetylcholinesterase activity

Inhibition of acetylcholine esterase (AChE) is indicated as a promising strategy in the treatment of PD. Most of the conventional drugs used have been reported to create severe adverse effects such as gastrointestinal disturbances, hepatotoxicity, nausea, syncope and bradycardia. So there a necessity of new approach that can prevent or limit the progression of disease and hence pharmacological research has focused on acetylcholinesterase inhibitors with fewer side effects in order to alleviate the cholinergic deficit and improve neurotransmission. Similarly, a considerable acetylcholinesterase inhibitor activity is expressed by *O. turpethum* roots extract (Figure 7). Vahedi-Mazdabadi et al. results revealed that aqueous extract of bitter apricot kernels showed potent *in vitro* acetylcholinesterase inhibitory and neuroprotective effects [26].

3.3 Total protein

Elevated TBARS levels indicate severe oxidative stress and accumulation of defective proteins in brain. Decrease in total protein by HEOT has proved its efficacy in alleviating oxidative stress induced increase in defective proteins by enhancing the antioxidant defensive mechanism (Figure 8). Goel and Choudhary study, measured the oxidative stress at various levels the defensive antioxidant enzymes in rat brain. LPO is the measure of the excessive oxidation of the lipids in the body indicating increased superoxide production. Therefore, the oxidative stress indices were estimated in rat brain such as LPO. The neuroprotective activity of the daidzein may be due to its antioxidant property, which reinforces anti-Parkinson's activity of daidzein [16].

3.4 Histopathological evaluation (Figure 9)

The histopathological study of Substantia Nigra (SN) in the rat brain showed that neurotoxins (positive control) caused marked hypertrophic changes, increased intracellular space, infiltration of neutrophils, decreased density of cells, alterations of architecture, haemorrhage, and neuronal damage and even cell death. Whereas the HEOT and reference standard L-Dopa & Carbidopa showed normal morphology (Figure 9). HEOT and standard drugs (Carbidopa + Levodopa) showed a significant neuroprotection and the same was proved by another study conducted by Goel and Chaudhary that also showed a significant neuroprotection by standard drugs (Carbidopa + Levodopa) and daidzein. [16]

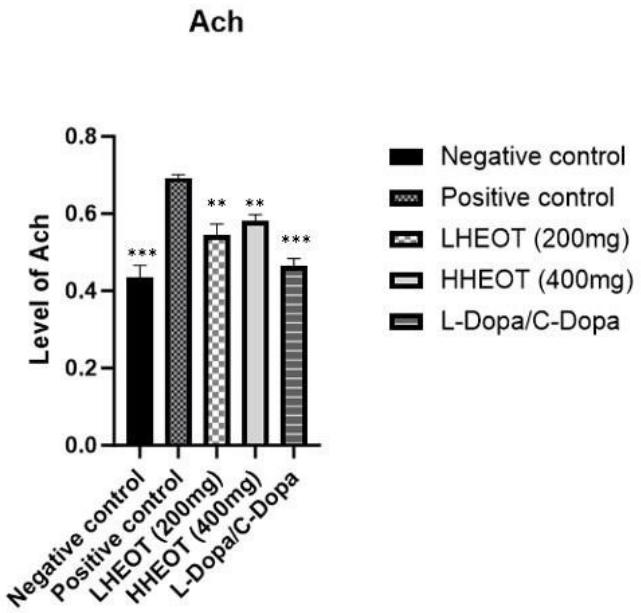

Fig 7. Graphical representation of HEOT effect on AChE levels

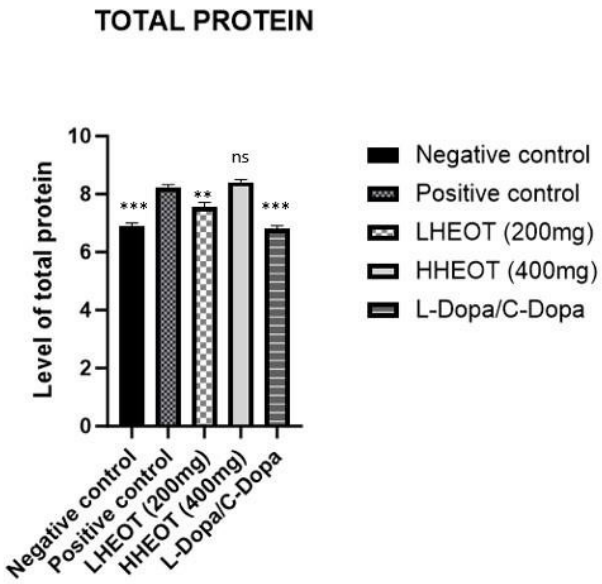

Fig 8. Graphical representation of the effect of HEOT on level of total protein

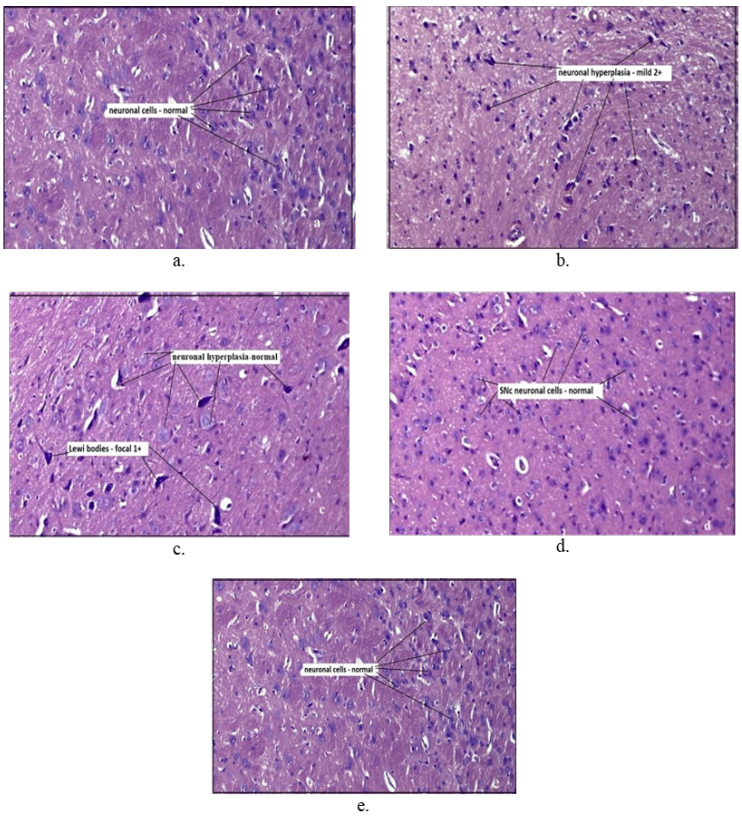

Fig 9. Histopathological findings of substantia Nigra (SN) region of rat brain. a. Negative control, Rat Brain: Substantia Nigra region showing normal morphology (X100); b. Positive control, Rat Brain: Substantia Nigra (SN) region showing neuronal hyperplasia mild 2+ (X100); c. low dose of HEOT, Rat Brain: Substantia Nigra (SN) region showing normal morphology (X100); d. high dose of HEOT, Rat Brain: Substantia Nigra (SN) region showing normal morphology, Lewi bodies – focal: 1+ (X100); e. Standard (L-Dopa & Carbidopa), Rat Brain: Substantia Nigra (SN) region showing normal morphology (X100)

The above behavioural and biochemical results suggest that *O. turpethum* extract has the ability to improve symptoms of Parkinsonism, in part, by restoring the level of dopamine, and by the regulation of the antioxidant system. Thus, antioxidant and neuroprotective activities may be responsible for antiparkinson's effect. Hence, *O. turpethum* extract may be useful as a neuroprotective agent in the treatment of PD.

4 Conclusion

Current therapeutic strategies for PD are mainly symptomatic and hampered by important side effects. It is therefore essential to promote the development of new therapeutic strategies aimed to interrupt or, at least, slow down the neurodegenerative process and thus the onset or progression of symptoms. Compounds able to positively modulate glial cells activity, reduce ROS levels and enhance autophagy may represent a valuable innovation in the treatment of PD. The *in vivo* and *in vitro* experimental results reported in the current study suggest that *O. turpethum* extract could be protective against the pathogenic processes implicated in PD. Its neuroprotective activity was comparable with that of L-dopa - carbidopa combination and thus future studies can be carried out in order to isolate the phytoconstituent that might increase dopamine levels in brain.

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