RESEARCH ARTICLE

Neuroprotective Effects of *Taraxicum Officinale* as an Antioxidant and Anti-neuroinflammatory Agent in Rotenone Induced Rat Model of Parkinson's Disease

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Received: 18th May, 2021; Revised: 9th June, 2021; Accepted: 20th July, 2021; Available Online: 25th September, 2021

ABSTRACT

Parkinson's disease is characterized by postural instability, rigidity, tremor, akinesia, uncontrollable shaking, and slow movement. Two main pathogenic processes cause it: oxidative stress and neuroinflammation. This study evaluates the effects of *Taraxicum Officinale* on rats via establishing a rotenone-induced model of Parkinsonism. This is the first study investigating the capability of *T. Officinale* to keep rats protected from rotenone-induced Parkinsonism via antioxidant and anti-inflammatory agents.

This case control study extended over three months and was carried out on 60 healthy male Albino rats, evenly divided into six groups: the first is the healthy control group, and the other five groups received rotenone 2.5 mg/kg IP every other day. The third, fourth, and fifth groups daily received oral doses of (300, 400, and 500 mg/kg) respectively of *T. Officinale* extract, and the sixth group daily received an oral dose of 10 mg/kg Sinemet.

Neurobehavioral analysis done via rotarod was performed on day 22. Then animals were decapitated, and brain tissue samples were prepared for homogenization to get the tissue supertant on which the biochemical tests were performed to measure the parameters of melanoaldehyde and nterleukin-1β.

Comparing the magnitude of *T. Officinale*'s effect with the rotenone group, the main results show that *T. Officinale* in groups 3, 4, and 5 boosted the motor coordination of the rats having Parkinsonism with a significant increase in rotation distance. This indicates that *T. Officinale* is good at improving symptoms of Parkinsonism in rats.

The main results of the biochemical analysis are *T. Officinale* groups 3, 4, and 5 show a significant decrease in melanoaldehyde and interleukin -1β levels compared with the rotenone group. In conclusion, it is experimentally approved that *T. Officinale* has an antiparkinson-like activity reversing the rotenone-induced Parkinsonism in male rats via anti-inflammatory and antioxidant activities.

Keywords: Antioxidants, Anti-neuroinflammatory agents, Parkinson's Disease, *Taraxicum Officinale*, Rotenone.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.3.1

How to cite this article: Baqir KA, Selman SM, Mohammed SB. Neuroprotective Effects of *Taraxicum Officinale* as an Antioxidant and Anti-neuroinflammatory Agent in Rotenone Induced Rat Model of Parkinson's Disease. International Journal of Drug Delivery Technology. 2021;11(3):649-655.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

After Alzheimer's disease, Parkinson's disease (PD) is the second most familial neurological disorder that mostly affects the mobility of old age people. Rigidity, tremor, akinesia, and postural instability are the most frequent symptoms produced by the massive loss of dopaminergic neurons that project from the substantia nigra (SN) to the striatum[1] is a complex, chronic, progressive, syndromal neurodegenerative illness that manifests clinically at the age of sixty. With 7–10 million Parkinsonians globally, PD is increasingly becoming a global community disease. By 2030, it is estimated that 8.7–9.3 million Parkinsonians would live in the world's top ten most populous countries.[2]

The PD is a frequent cause of morbidity that affects 1–2 per 1000 people at any time, with the elderly being the most affected. Furthermore, men are somewhat more affected than women. There may be an increase in disease recurrence that cannot be explained just by population demographic changes. The clinical diagnosis accuracy is thought to increase as a result of the revised diagnostic criteria. Increased knowledge of PD genetic risk factors, together with data on environmental risk factors, will almost certainly lead to a superior understanding of the disease's origin in the near future.[3]

Both oxidative stress and neuroinflammation have been linked to the etiopathogenesis of PD. The two central pathways in microglial cell activation that contribute to progressive

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neuronal degeneration and provide a promising therapeutic target in PD are oxidative stress and neuroinflammation. The activation and release of pro-inflammatory cytokines including interleukin 1-β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor (TNF-α), and also the production of free radicals like reactive oxygen species (ROS), reactive nitrogen species (RNS) and inducible nitric oxide synthase (iNOS), have a negative impact on the surviving of DA-ergic neurons in the SNc.[4]

Neurotoxins and genetics are the two common primary models utilized to simulate PD in experimental animals.[5] Rotenone is the most widely used environmental pesticide that has been related to an increase in the prevalence of PD. Rotenone is a lipophilic and very effective complex I inhibitor that causes the death of DA-ergic neurons and, as a result, motor deficits in PD.[6]

A balance between pro- and antioxidant systems is usually essential to maintain cellular homeostasis. As a result, one of the newest trends is the use of antioxidants to restore the cellular antioxidant system which act as therapeutic strategies to protect vulnerable DA-ergic neurons from oxidative stress and inflammation that results.[4]

Surprisingly, the World Health Organization (WHO) promotes traditional medicinal herbs for treatment and prevention. However, no experimental investigation on the preventive potential of *T. Officinale* against rotenone-induced neurotoxicity in rats as an experimental model of PD has been done so far. *T. Officinale* (also known as Dandelion) was chosen for this study because it is part of the Compositae family (Asteracea), a perennial herb typically viewed as a weed that can be found almost anywhere. It has antioxidant properties and radical scavengers, enzyme inhibitors, metal chelators, hydrogen donors, and singlet oxygen quenchers. *T. Officinale* possesses strong radical scavenging activity and antioxidant capacity due to its greater phenolic and flavonoid content. *T. Officinale*'s antioxidant properties are transferred into tissue antioxidant status.[7] Thus, this study aims to evaluate the effects of T. Officinale on rats via establishing a rotenone-induced PD model.

MATERIALS AND METHODS

Animals

This experiment enrolled sixty male adult Albino rats whose weights range between 200–300 grams. The rats were housed in the Animal House of the College of Medicine, University of Babylon. They were kept on twenty-five centigrade temperatures with fourteen hours in daylight and ten hours in darkness cycle with water and food ad libitum. The animals were randomly divided into six groups after two weeks of adaptation. According to the experiment. The study was done at the College of Medicine, University of Babylon, from 1 Oct, 2020 to 1 March, 2021.

Plant Preparation

The dried leaves and roots of the plant inwas approved to be *T. Officinale* with the help of College of Agriculture, Medicinal Plant Department, Al-Qasim Green University according to document No. 2387 on 3/11/2020.

To determine the yield of the extract, the dried leaves and roots were cleaned, sorted, ground into a powder using a pestle and mortar, and weighed. Hydro ethanol (70% ethanol solvent) was used to extract the powdered leaves and roots, which were then shaken for 72 hours on an automated shaker. With a Buchner funnel and Whatman No. 1 filter paper, the mixtures were filtered. Ethanol was recovered from the filtrates using a rotary evaporator at 40°C and decreased pressure. In a fan oven set to 40°C, the water was removed. The study was conducted using a dry extract.[7] Extracts were diluted with water, shortly before the experiment, to the final concentrations.[8] Then 5 gm of the dried extract was dissolved in 20 mL DW and the final product contained 500 mg in each mL.

Rotenone Preparation

ROT (2.5 mg/kg BW) was given IP to rats to induce PD. ROT was initially dissolved in a 50X stock solution of dimethyl sulfoxide (DMSO)[9] 125 mg rotenone dissolved in 1 mL DMSO The stock solution was then diluted in 1960 µL of olive oil with 40 µL of the stock solution., fresh solution was prepared twice a week, the solution was vortexed to obtain a uniform mixture before administration to rat. Each rat was given 1-mL/kg of prepared solution, while the control group of animals were given simply the vehicle (olive oil/DMSO).[10]

Study Design

The sixty rats were randomly divided into six groups, ten animals per each as follow:

- Group I: healthy control group.
- Induce parkinsonism in the rest fifty rats by rotenone IP 2.5 mg/kg every 48 hours (every other day) for 21 days[11] and subdivided as follow:

a) Group II: untreated PD rats
b) Group III: 300 mg/kg of *T. officinale* daily for twenty-one days orally by a gavage
c) Group IV: 400 mg/kg of *T. officinale* daily for twenty-one days orally by a gavage
d) Group V: 500 mg/kg of *T. officinale* daily for twenty-one days orally by a gavage
e) Group VI: 10 mg/kg of sinemet tablet daily for twenty-one days orally by a gavage

Twenty-four hours after the last dose, at day 22, behavioral tests were performed to compare parkinsonism development and treatment effectiveness. Each animal was placed on the rotarod for three trials and all behaviors were recorded by video camera.

Then, each animal was sacrificed by cervical dislocation to get midbrain samples to measure tissue levels of IL-1β and MDA.

Behavioral Test

The rotarod test is used to analyze rodents' motor coordination and capacity. It necessitates rats balancing on a rotating cylinder whose speed can be adjusted.[12] Each rat was put on the revolving cylinder (speed: 20 rpm) and examined for

three mint. The distance of rotation was recorded to score the performance of rat for their motor coordination. The device was cleaned with 10% ethanol after each try[13].

Brain Dissection

On 22th day, each rat was sacrificed, and the brains were removed after dissection of the skull from foramen magnum posteriorly. Cerebellum and olfactory pulps were removed, and the brain was gently removed from the skull, and the mid and forebrain were taken and dissected out and rinsed with phosphate buffer solution and weighted.

Steps of Preparation of Brain's Samples

- Brain homogenization: residual blood was removed by washing with pre-cooling PBS buffer (pH = 7.4).
- Brain was homogenized after weighing, then it was homogenized in PBS (pH = 7.4) with a homogenizer on ice.
- Thawing at 2–8ºC or freezing at -20ºC.
- After thawing, the homogenates are then centrifuged at 2000–3000 RPM for 20 minutes.

Assessments of MDA and IL-1β using ELISA Kits

MDA and IL-1β were measured by enzyme-linked immunosorbent assay (ELISA).

Statistical Analysis

The results of the study are statistically tabulated using the SPSS. The statistical equations used to find the significant differences are One-way ANOVA and the post hoc test.

RESULTS

Weight Results

In group 1 (control group, untreated and unexposed to rotenone), there were no significant differences ($p>0.05$) in the means of weight on day 10 and day 21 as compared with day 0 (Table 1 and Figure 1).

Table 1: Comparison of means of weight ± SEM between groups on days 0, 10, 21

Wt	*Group 1*	*Group 2*	*Group 3*	*Group 4*	*Group 5*	*Group 6*
Day 0	252.300 ± 15.19653	277.300 ± 14.21662	240.500 ± 17.92407	240.500 ± 16.65746	258.800 ± 9.08699	258.600 ± 15.39856
Day 10	257.700 ± 16.32997	237.600 ± 13.16409	215.400 ± 18.49156	198.600 ± 5.77196	216.200 ± 9.83734	246.400 ± 15.35665
Day 21	269.3 ± 15.97780	217.9 ± *12.19695	216.7 ± *14.07444	184.9 ± $^{\Omega}$*6.57850	190.1 ± $^{\Omega}$*8.86999	234.700 ± 15.02594

$^{\Omega}$ = significantly decreased (p value <0.05) as compared with day 10.
* = significantly decreased (p value <0.05) as compared with day 0.

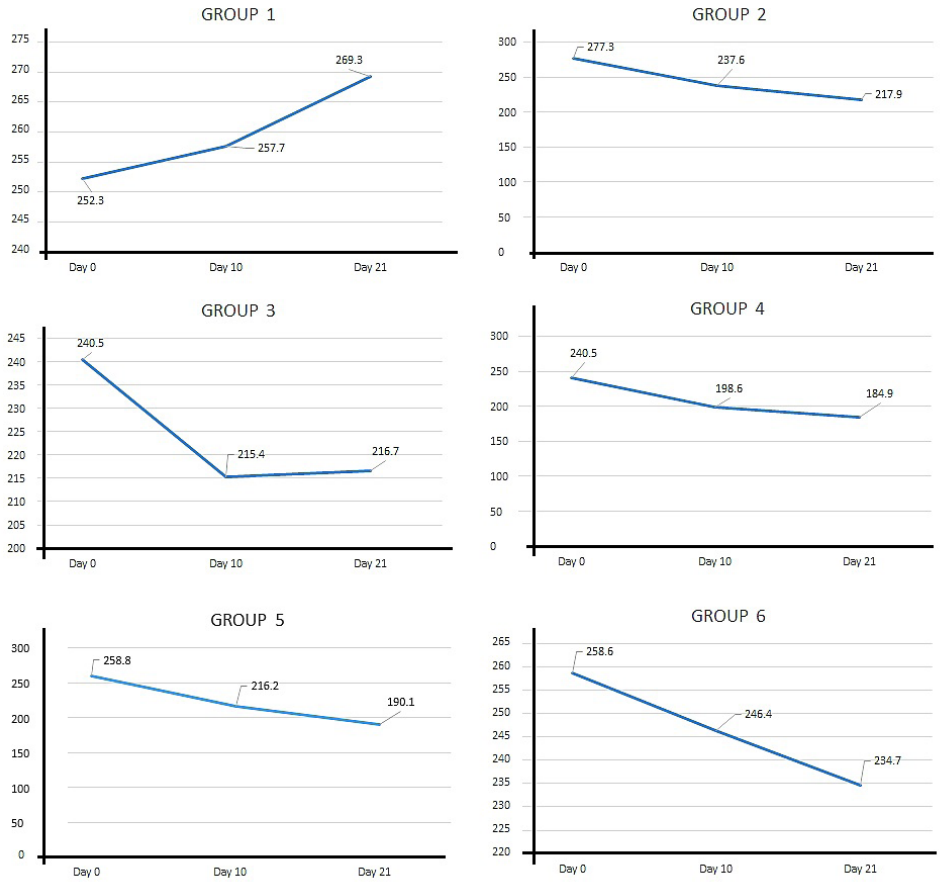

Figure 1: The means of body weights on days 0, 10, 21 for all groups.

Furthermore, in group 2 (untreated but exposed to rotenone), the means of weight were significantly decreased ($p < 0.05$) on day 21 as compared with day 0 (Table 1 and Figure 1).

In group 3 (treated with 300 mg/kg *T. Officinale* and exposed to rotenone), group 4 (treated with 400 mg/kg *T. Officinale* and exposed to rotenone), group 5 (treated with 500 mg/kg *T. Officinale* and exposed to rotenone) the means of weight were significantly decreased ($p < 0.05$) on day 21 as compared with day 0, while group 4 (treated with 400 mg/kg *T. Officinale* and exposed to rotenone) and group 5 (treated with 500mg/kg *T. Officinale* and exposed to rotenone) have shown significant decrease ($p < 0.05$) in the means of weight on day 21 as compared with day10 (Table 1 and Figure 1).

In group 6 (treated with Sinemet and exposed to rotenone) there were no significant differences ($p > 0.05$) in the means of weight on day 10 and day 21 as compared with day 0 (Table 1 and Figure 1).

Group 1 (control group, untreated and unexposed to rotenone), group 2 (untreated and exposed to rotenone), group 3 (treated with 300 mg/kg *T. Officinale* and exposed to rotenone), group 4 (treated with 400 mg/kg *T. Officinale* and exposed to rotenone), group 5 (treated with 500 mg/kg *T. Officinale* and exposed to rotenone) and group 6 (treated with Sinemet and exposed to rotenone), no. of rat = 10 rats for each group.

Group 1 (control group, untreated and unexposed to rotenone), group 2 (untreated and exposed to rotenone), group 3 (treated with 300 mg/kg *T. Officinale* and exposed to rotenone), group 4 (treated with 400 mg/kg *T. Officinale* and exposed to rotenone), group 5 (treated with 500 mg/kg *T. Officinale* and exposed to rotenone) and group 6 (treated with Sinemet and exposed to rotenone), no. of rat = 10 rats for each group.

Rotarod Test

Rotations distance significantly decreased ($p < 0.05$) in group 2, group 3, group 4 and group 6 as compared with group 1, while the rotations distance insignificantly decreased ($p > 0.05$) in group 5 as compared with group 1. Moreover, the rotations distance significantly increased ($p < 0.05$) in group 3, group 4, group 5 and group 6 as compared with group 2 (Figure 2).

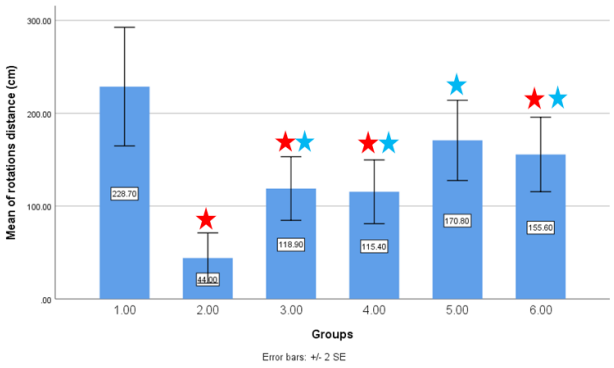

Figure 2: Means of rotations distance (cm) ± SEM of all groups.
= significantly decreased ($p < 0.05$) as compared with group 1.
= significantly increased ($p < 0.05$) as compared with group 2.

Malondialdehyde Levels

Malondialdehyde (MDA) levels significantly increased ($p < 0.05$) in group 2 and group 6 as compared with group 1 while significantly decreased ($p < 0.05$) in group 6 as compared with group 2 (Figure 3).

MDA levels significantly decreased ($p < 0.05$) in group 4 and group 5 as compared with group 3 (Figure 3).

Interleukin -1β Level

Interleukin-1β (IL-1β) levels significantly increased (p-value <0.05) in group 2 and group 6 as compared with group 1, while significantly decreased (p-value <0.05) in group 3, group 4 and group 5 as compared with group 2 and group 6 (Figure 4).

Also IL-1β was significantly decreasing ($p < 0.05$) in group 4 and group 5 as compared with group 3 while significantly increased (p-value <0.05) in group 3 as compared with group 1 (Figure 4).

DISCUSSION

Although the exact mechanism behind the pathophysiology of PD is still unknown, oxidative stress and neuro-inflammation

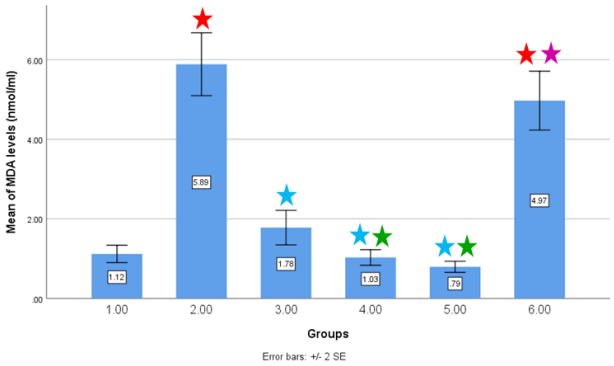

Figure 3: Means of MDA levels (nmol/mL) ± SEM of all groups.
= significantly increased ($p < 0.05$) as compared with group 1.
= significantly decreased ($p < 0.05$) as compared with groups 2 and 6.
= significantly decreased ($p < 0.05$) as compared with groups 3.
= significantly decreased ($p < 0.05$) as compared with groups 1.

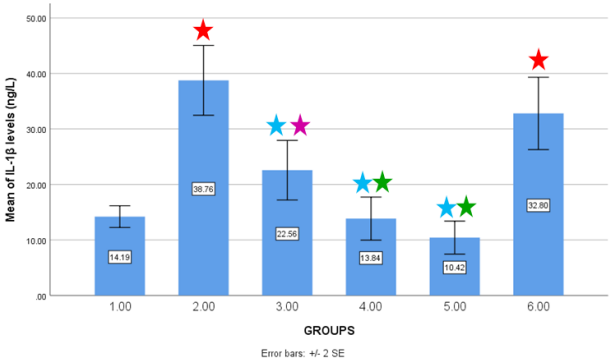

Figure 4: Means of IL-1β levels (ng/L) ± SEM of all groups.
= significantly increased ($p < 0.05$) as compared with groups 1, 3, 4, 5.
= significantly decreased ($p < 0.05$) as compared with group 2 and 6.
= significantly decreased ($p < 0.05$) as compared with group 3.
= significantly increased ($p < 0.05$) as compared with group 1.

have been closely linked to the death of dopaminergic (DAergic) neurons in the SNc, which leads to striatal DA reduction.[14] Moreover, parkinsonism has also been linked to a lack of striatal DA and direct assault on striatal neurons.

Despite some important discoveries from mechanistic investigations, there is still no cure for Parkinson's disease. However, developing a therapeutic approach that can slow or stop disease development is restricted to small-scale clinical trials or animal experiments.[15]

T. Officinale has a neuroprotective effect and may help protect the brain from oxidative damage. Hence, this study was designed to investigate the antiparkinson effect of *T. Officinale* on rats via establishing a rotenone-induced model of PD.

In comparing group 2 (untreated and exposed to rotenone) with group 1 (control), it is found that the former reveals a significant decrease in the rat's weight on day 21 as compared with day 0. Prior to treatment, each rat's weight was assessed, and then every week after that. Throughout the study, the average weight of the rats in the control group grew. Rats in the rotenone group, on the other hand, lost weight, which was thought to be linked to gastrointestinal neuron damage,[16] a matter which impeded digestion. Patients with Parkinson's disease also reported weight loss as a symptom. The weight loss recorded in this study is consistent with the results published,[17] and may be caused by the rats' acute in adaptation to the rotenone treatment.

When the rats were given rotenone, they became extremely weak and had trouble moving and eating. They also showed behavioral deficiencies due to the treatment, including weight loss, impaired motor abilities, and decreased food intake.[18] Early studies linked the depletion in DA level with decreased movement, leading to lesson food consumption and thus causing a decrease in body weight.[19]

After *T. Officinale* treatment, the significant weight reduction is recorded in Parkinson's affected rodents on day twenty-one as compared with day zero for 300 mg, 400 mg and 500 mg/Kg plant doses and on day twenty-one as compared with day ten for 400 mg and 500 mg/Kg plant doses. The weight reduction in these groups is higher in rotenone group due to rotenone, considered to be linked to gastrointestinal neurons damage. Moreover, *T. Officinale* root and leaves supplement have improved plasma antioxidant enzymes activity and lipid profiles in cholesterol-fed animals, suggesting that it may have hypo-lipidaemic and antioxidant properties.[20] According to flavonoids found in *T. Officinale*, inhibiting pancreatic lipase, a key enzyme in fat digestion, could be an effective means to alter fat absorption. *T. Officinal*e leaves, such as luteolin and others, may lower pancreatic lipase activity.[21]

There was no significant decrease in the weight of day 10 and 21 in Sinemet group compared with day 0. This insignificant decrease in weight is due to the effect of rotenone overcome by L-dopa that compensates for the diminished DA levels.

In the rotarod apparatus, repeated rat exposure to rotenone significantly lessened the coordination of muscles (rotations number, rotations distance, and time of rotations) compared to the control group, which agrees with the previous studies.[22] The decrease in the DA level is linked to decrease movement in some previous studies.[19]

T. Officinale treatment preserved the coordination of muscles in the rotarod apparatus and significantly increased rotation distance as compared to group 2. This indicates that *T. Officinale* has a good effect on improving PD symptoms in rats, especially with a 500 mg/kg dose, which showed an insignificant difference compared to the control group. This finding by the current study has not been arrived at by previous studies, as far as the researcher could investigate. This improving effect of *T. Officinale* is attributed to its powerful neuroprotective impact that has shown an efficient outcome as a neuroprotective agent in other neurodegenerative diseases as Alzheimer's disease because of retaining its anti-inflammatory and antioxidant agents.[23] Some studies have attributed the efficiency of the *T. Officinale* crude extractions in preventing ROS-induced loss to chlorogenic and caffeic acids, in addition to the flavones luteolin and luteolin 7-*O*glucoside.[21]

Major progress of the rotarod performance on day 21 resulted from the typical treatment of L-dopa and carbidopa compared with animals treated with rotenone due to increasing DA level, which agrees with other studies.[24]

The current study has found that the rotenone group has shown a highly significant increase in the MDA level as compared with the control group, which agrees with other study.[25]

The development of neurodegenerative disorders is influenced by oxidative stress, which has been associated with PD development in both pre-clinical and clinical investigations, especially elevation the concentrations of oxidative markers such MDA. Similarly, pre-clinical investigations clearly demonstrated that oxidative stress in PD is caused by environmental factors such as neurotoxins, insecticides, pesticides, and DA itself. Pesticides, such as rotenone, have been shown to enhance ROS by blocking mitochondrial complex I activity, resulting in oxidative stress, which may cause SNCA accumulation.[26]

In Parkinson's disease-affected rat groups treated with *T. Officinale* (300, 400, and 500 mg/kg), there is a considerable reduction in MDA levels compared to the rotenone with respect to lipid peroxidation antioxidant concentration, indicating that the plant has antioxidant capacity. The decrease of ROS formation from the primary caffeoyl polyphenols derivatives could explain the antiperoxide activity seen in *T. Officinale*-treated animals. Free radicals, such as hydroxyl anions, can be scavenged by chlorogenic acid, cichoric acid, and total caffeoyl polyphenols derivatives.[27]

Some studies demonstrated that enzymatically induced lipid peroxidation has been reduced by ethyl alcohol (EtOH). and showed scavenging activities against ROS and RNS, which were contributed to the existence of phenols in the EtOH and were shown to have antioxidant activity and neuroprotective effect and preferred on the watery extract.[28] Thus, oxidative stress inhibition could be one of the mechanisms underlying *T. Officinale's* anti-Parkinson benefits.

There was also a significant increase in multiple discriminant analysis (MDA) level in Sinmet group compared with the control group but less than rotenone group.[29]

Treatment with L-dopa resulted in a rise in MDA and oxidized glutathione (GSH) levels, in addition to a depletion in reduced GSH. This impact could be explained by the fact that recurrent L-dopa treatment increases DA synthesis, leading to an excess of free radical generation, which would overwhelm the endogenous defense mechanism, causing an excess of oxidative stress. Previous investigations have shown that repeated L-dopa administration causes oxidative stress and inflammation, which supports some theories.[30]

This study has found that the rotenone group has shown a highly significant increase in the IL-1β level compared with the control group. This rise indicates that rotenone causes microglia and astrocytes to become activated, increasing the expression of pro-inflammatory markers such TNF-α, IL-6, and IL-1β in rats and promoting numerous inflammatory cascades. The loss of neuronal activity caused by these inflammatory cascades shows that neuroinflammation is important in neurodegenerative diseases, including PD, which agrees with the previous studies.[31]

In chronic neurodegenerative disease, IL-1β is associated to (a) microglia activation, (b) IL-1β induction, and (c) neurotoxicity and neurodegeneration as a result of induction of neuroinflammation. Neurotoxins have three modes of action: direct, indirect, and mixed. Neurotoxicity is caused by direct neurotoxins that do not activate microglial cells. Indirect neurotoxins can cause neurotoxicity when microglial cells are activated. Rotenone is an example of a neurotoxin that can produce neurotoxicity in two ways: directly and indirectly. Microglia NF-κB activation and, as a result, microglia-induced production of IL-1 contribute to neurotoxicity, according to the mixed-mode category of rotenone. As a result, the microglial mechanism of NF-κB activation-induced IL-1β production offers much therapeutic promise. Pro-inflammatory substances like IL-1β, oxidative stress products, and PGs are all probable pathways from active microglia to neurotoxicity.[32]

After *T. Officinale* treatment, a significant reduction in IL-1β level is produced in Parkinson's affected rat groups (300, 400, 500 mg/kg) compared with rotenone group. *T. Officinale* is found to inhibit TNF-α production from rat astrocytes by decreasing IL-1β production and exhibited anti-inflammatory effects in the CNS. The most effective anti-inflammatory activities of *T. Officinale* leaves were due to downregulation of NO, PGE2, and pro-inflammatory cytokines and decreased expressions of iNOS and COX-2. The main components responsible for the anti-inflammatory effect are luteolin and cichoric acid by inhibiting the phosphorylation of NF-κB pathways. The third component, taraxasterol, has an anti-inflammatory impact by blocking the NF-κB pathway, which up-regulates inflammatory enzymes like iNOS and COX-2 and pro-inflammatory cytokines like TNF-α and IL-1, when NF-κB is activated. NF-κB is thought to be a critical target for anti-inflammatory therapies.[33,34]

There is also a significant increase in IL-1β level in Sinemet group compared with the control group but slightly less than rotenone group.[35] This increase is due to the effect of rotenone in activating microglia and astrocytes.

CONCLUSIONS

The main conclusion of this study is that *T. Officinale* is an effective and safe plant that attenuates the signs of PD. This conclusion is arrived at by the study's findings, which have shown that this plant is effective in decreasing level of MDA and the concentrations levels of IL-1β in brain tissues. Thus, it is highly recommended that *T. Officinale* is used as a food supplement by Parkinson's patients. Besides, its extract can be utilized as a co-treatment with anti-Parkinson drugs.

Finally, it is advisable to investigate further higher levels of concentration of *T. officinale* extract and/or employing a bigger number of rats with a longer duration of experimental time and further identification of its major active constituents.

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