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# Modafinil Alleviates Rotenone- Induced Neurochemical Changes and Striatal Nerurodegeneration via Inhibiting Oxidative Stress and Neuroinflammation

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**Abstract**

Objectives of study are around investigation the effect of the central nervous system stimulant modafinil on brain striatum neurodegeneration caused by subcutaneous (s.c.) rotenone administration in rats, further, the possible modulation by modafinil on L-dopa effect on oxidative stress, inflammation and nigrostriatal cell damage. Seven groups of Male albino mice received dimethyl sulfoxide s.c., rotenone (1.5 mg/kg, s.c., 3 times per week), rotenone/L-dopa (25 mg/kg, p.o., daily), rotenone/modafinil (0.1, 0.2 and 0.3 mg/kg respectively, p.o., daily), rotenone/L-dopa/modafinil (0.2 mg/kg, p.o. daily). The treatment was continued for 2 weeks. Mice were tested for behavioral changes 24h after the end of treatments.Mice were evaluated for brain biochemical markers of oxidative stress (lipid peroxidation, reduced glutathione, and nitric oxide), pro-inflammatory factors (tumor necrosis factor-α, interlukin-1beta) and dopamine level. Histopathologic examination and the expression of the anti-apoptotic protein caspase-3 were also performed. Rotenone significantly elevated oxidative stress and pro-inflammation, decreased dopamine, induced substantia nigra damage with caspase-3-mediated apoptosis respectly to the control levels. Results of modafinil or its combination with L-dopa may have potential therapeutic effect in Parkinson's disease by decreasing pro-inflammation and oxidative stress, increasing dopamine, preventing the development of neuronal damage and reducing caspase-3 expression in the striatum.

**Keywords***:* Modafinil, Brain oxidative stress, neuro-inflammation, apoptosis, nigrostriatal damage, rotenone, L-dopa.

## Introduction

**Parkinson's disease (PD) is considered as the second most prevalent neurodegenerative disorder worldwide, 1% of people over 65 years nearly were affected [1]. The output of the basal ganglia in PD, is irreversibly affected due to degeneration of the neuromelanin-containing dopaminergic neurons in the substantia nigra pars compacta (SNc) and subsequent striatal dopamine depletion [2]. This results in the manifestation of symptoms including bradyakinesia or slowed movement, gait and postural instability, muscle rigidity and resting tremors [3]. In most cases, the etiology of the disease remains elusive. Although the exact reason of Parkinson's disease is not yet recognized, it is considered to be potentiated by the interaction of environmental, genetic and oxidative factors [4]. Fewest amount of pesticides,**

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especially that containing manganese, exposure and using well water supply in rural areas can greatly raise the risk of PD [5]. 5% to15% of cases may be due to genetic causes that having young age of disease onset and are due to either mutations in the gene SYN (A30P and A53T) or in PINK1 gene leading to the appearance of PD. While SYN mutation results in internal cellular α-synuclein to accumulate and deposit to form Lewy bodies, dopaminergic neuronal loss, and motor function deficit, which are hallmarks of PD , the PINK1 gene appears to have a different mechanism that is interlaced with the oxidative stress risk factor [6,7]. The increases of norsalsolinol, an endogenous neurotoxin present in dopamine-rich areas that influenced cytochrome c release and caspase 3 activation, and deficiency in MTH1, an oxidized purine nucleoside triphos-phatase that suppresses cell death caused by oxidative stress, are strongly participating to the increase of ROS from oxidative defeats playing a significant role in PD [8.9].

In rodents, one pesticide which is rotenone was proved to reproduce several pathologic features of PD, including nigrostriatal dopaminergic cell degeneration and alpha-synuclein-like positive cytoplasmic inclusions [10,11]. Levodopa (L-dopa) (L-3,4- dihydroxyphenylalanine) since its introduction in 1960s, L-dopa has remained the main dopamine replacement effective treatment in controlling the symptoms of PD and is associated with the greatest improvement in motor function [12,13]. Now a day, despite improved control of symptoms with levodopa (L-dopa) and dopamine agonists, patients are often disabled and respond poorly to medications [14]. Also, different dopaminergic drugs including L-dopa do not counteract the progressive nature of the disease, neuronal cell death continues and long-term treatment is often associated with declining efficacy and an increase in side effects [15]. This is why, in PD, there is an immense need to get a more better strategy for prevention of the neuronal death, stopping or slowing down the progression of neurodegeneration . Therefore, the aim is to focus on getting neuroprotection via pharmacological interference with the aim to increase neuronal survival. Although some potential drug candidates were tested in clinical trials there is as yet no proven neuroprotective treatment [15]. Modafanil is a vigilance-stimulating compound used for the treatment of narcolepsy and approved by the Food and Drug Administration (FDA) [16]. Later, increasing evidence indicated the effectiveness of the drug in treating several disorders such as, fatigue, cocaine addiction, nicotine addiction, attention deficit disorder, depression, seasonal affective disorder, bipolar depression. Some clinical trials also indicated a possible use in the treatment of neurodegenerative diseases like schizophrenia [17].

The mechanism of action of modafinil is not clear but research on modafinil's wake-promoting mechanism revealed that modafinil increases indirectly wakefulness via alpha-1 noradrenergic neurotransmission [18]. It also decreases gaba-amino- butyric acid (GABA) release in sleep-related areas and striatum [9,20], stimulates histamine, norepinephrine, serotonin, dopamine (DA), and orexin systems in the brain [17]. Mignot et al [21] find that modafinil has more potent behavioral effects than some molecules that bind with a much greater affinity to the dopamine reuptake transporter, whereas knocking out the DA transporter prevents the simulative properties of modafinil [21,22].

In the neurodegenerative process that causes PD, factors like mitochondrial dysfunction, excitotoxicity, oxidative stress, inflammatory processes, and disturbed calcium homeostasis either separately or cooperatively, is involved [23]. There have also been a number of researches which proved that modafinil could also be very promising as an antioxidant compound and neuroprotective. Modafinil could directly reduce free-radical levels through acting on enzymes in the brain's free-radical scavenging system (e.g., glutathione peroxidase or superoxide dismutase). Modafinil has an ability to increase the cortical creatine-phosphocreatine pool by targeting the mitochondria the main source of reactive oxygen species in the cell, thuspromoting ATP production and inhibiting free-radical production, which increase creatine-phosphocreatine production [24]. Modafinil may enhance cytochrome c ability to accept and donate electrons by catalytic mechanism orallosteric modification and this would directly diminish net hydrogen peroxide levels and superoxide production and increase ATP production. This mechanism would also involve reduced activity of the inhibitory ATP sensitive potassium channels ($K_{ATP}$-channels) that inhibit neurotransmitter release and so, it account for increased neurotransmitter release [25]. Modafinil may adjust adenosine levels and also, play an antioxidant role all over the entire brain, but it is in the basal forebrain that a reduction in adenosine resulting from reduced reactive oxygen species levels would have its maximum wake-promoting effects [26]. Modafinil's effects on neurocognitive functioning through increasing blood flow which lead to changes in cortical activation and improved whole brain function rather than localized neural excitation [27].

Therefore, the present study focuses on putative neuroprotective and antioxidant effects of modafinil in nigrostriatal cell apoptosis/injury in the mice PD model induced by the systemic administration of the pesticide rotenone, with conducting behavioral tests, biochemical measurements of oxidative and nitrosative stress, neuroinflammation, histopathology and caspase-3 immunohistopathology.

## Materials and methods

### Animals

The study was conducted on 70 male albino miceaged 5–6weeks and of body weight 22–25 gm.they were maintained as performed by national guidelines and protocols, approved by the National Research Centre Ethics Committee (Publication No. 85-23, revised 1985). They were housed in clean and disinfected cagesand standard laboratory food and water were provided freely and were subjected to natural photoperiod of 12 h light: dark cycle throughout the experimental period (3 weeks).

### Drugs and chemicals

Rotenone,L-dopa (SinemetR tab (carbidopa/l-dopa, 25/250), dimethyl sulfoxide (DMSO) (Sigma–Aldrich Chem. Co, MA, USA), Merk& Co. Inc., Whitehouse Station, NJ, USA), modafinil (Bravamax; Chemipharm Pharmaceutical Industries, Egypt) were used in the study.

### Experimental design

Animals were divided into seven groups (10 mice each). Group (1) which is a control group that received dimethyl sulfoxide (DMSO) S.C. Group (2) mice was treated with rotenone for induction of parkinsonian behavior (1.5

mg/kg, S.C, 3 times per week, dissolved in DMSO)[25]. Group (3) received rotenone concomitantly with L-dopa (25 mg/kg, daily, orally) [26]. Groups 4, 5 and 6 were treated with rotenone concomitantly with modafinil orally at doses of 0.1, 0.2 and 0.3 mg/kg respectively,daily. Group (7) received rotenone/L-dopa/modafinil orally (0.2 mg/kg daily). The treatment regimen was continued for 2 weeks in the 7 groups. Behavioral tests were performed (wire hanging, stair, and wooden rod test) after twenty four hours of the treatment. Then the mice were sacrificed by decapitation and their brains were dissected on ice-cold plate. The striatum of each brain was taken out, washed with ice-cold saline (0.9%), weighed, and stored at −80 °C till the determination of PD related biochemical tests. The brain was homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10% w/v for the biochemical assays. Part of the harvested brains was kept in 10% formula saline for histopathological investigations.

**Neurobehavioral testing:**

All mice were screened for motor behavioral injury using the wire hanging, stair, and wooden walking testat the end of the experimental.

**Wire hanging test**

Wire hanging test was done to evaluate the Neuromuscular strength, where mouse was placed with its forelimbs on a wire mounted horizontally of 20 cm length, 50 cm above the surface. 30 seconds cut off time was taken for the latency time to fall which was recorded. To avoid injury of the mice soft padding was placed on the landing area [27].

**Wood walking test**

Mice were made to walk over a wooden stick (~1 m in length, 1 cm in width) and the time each mouse spent to reach the end is recorded in order to test the behavioral (wire hanging, stair, and wooden rod test) [28].

**Stair test**

Mice were placed at the bottom of a stair (30 cm in length) placed at an angle of 55° above the bench, and the latency to climb the stair is recorded for each mousein order to assess skilled reaching [29].

**Biochemical analysis**

**Oxidative stress markers**

In brain samples, Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Placer et al. [30] and the results were expressed as nmole malondialdehyde (MDA)/g wet tissue. Brain glutathione (GSH) was measured according to Ellman [31] and expressed as µmol/g wet tissue. In addition, nitric oxide was measured as nitrite which determined by using Griess reagent [32], according to Moshage et al. [32]method.

**Brain dopamine**

Brain dopamine (DA) was determined by (Competitive ELISA) which is a 1.5 hour solid-phase ELISA designed for the quantitative determination of Rat DA.

**Brain cytokines**

Brain IL-1β and TNF-α were evaluated by enzyme linked immunosorbent assay (ELISA) according to the methods of Kitaura et al. [33] and Tamaoki et al. [34], respectively, using commercial ELISA kits (In vitrogen Corporation Camarillo, California, USA) and microtiter plate reader (Fisher Biotech, Germany). An aliquot of sample or calibrator containing the antigen to be quantified is allowed to bind with a solid phase antibody. After washing, enzyme labeled antibody is added to form a sandwich complex of solid phase Ab-Ag-Ab enzyme. Excess (unbound) antibody is then washed away, then enzyme substrate is added.The enzyme catalytically converts the substrate to product, the amount of which is proportional to the quantity of antigen in the sample.

**Histopathological evaluation**

Brains were fixed in 10% neutral-buffered formalin, processed, and embedded in Paraffin blocks. Five-micrometer thickness sections were prepared for both routine H&E staining.

**Immunohistochemistry**

Paraffin-embedded specimens were cut into 4-mm sections. After deparaffinization with xylene and rehydration, antigen retrieval was performed by microwave treatment in 10 mmol l-1 sodium citrate buffer (pH 6.0) for 20 min. To block the endogenous peroxidase we used 3% $H_2O_2$ in methanol. Protein-blocking buffer was used to block non-specific binding for 10 min using. Phosphate-buffered saline (PBS) was used to wash the sections. We added diluted primary antibodies against Caspase 3 (Thermo Fisher Scientific Tudor Road, Manor Park, Runcorn, Cheshire WA7 1TA, UK) to the tissue and incubated overnight at 4ºC. normal mouse serum were added in the negative controls instead of the primary antibody. Human tonsil was used as positive control. Then samples were incubated with the horseradish peroxidase labeled secondary antibody (Thermo Fisher Scientific Tudor Road, Manor Park, Runcorn, Cheshire WA7 1TA, UK) for 30 min at room temperature. Diaminobenzidine was used for colour development and hematoxylin as counter stain.

**Statistics**

Results are expressed as mean ± SE. The results of the biochemical assays were analyzed using One Way ANOVA followed by Tukey's multiple comparisons test for multiple group comparison. Data of the behavioral study were analyzed by Kruskal-Wallis test followed by Dunn's multiple comparisons test. Graph Pad Prism software, version 6 (Graph Pad Software, Inc., San Diego, USA) was used for the statistical analysis. A probability value of less than 0.05 was considered statistically significant.

## Results

**Biochemical results**

**Oxidative stress markers:**

**Lipid Peroxidation**

Rotenone caused significantly increased MDA concentrations in the striatal tissue than those of the control-treated group. MDA increased by 304.02% (127.47 ± 4.52 vs. 31.55 ± 1.25 nmol/ g tissue). Administration of modafinil at doses of 0.1, 0.2 and 0.3 mg/kg decreased MDA by 45.64%, 53.67% and 57.87%, respectively, compared with the rotenone control group (69.33 ± 3.84, 59.1±4.17 and 53.73 ± 1.37 vs. rotenone value 127.47 ± 4.52 nmol/ g tissue). Also administration of L-

dopa alone or in combination with modafinil decreased MDA by 45.15% and 62.89%, respectively, compared with the rotenone group (69.97±4.82 and 47.34±3.48 vs. 127.47 ± 4.52 nmol/ g tissue) (Fig.1).

**GSH**

Compared with vehicle-treated mice, the rotenone group exhibited 70.25% decrement in GSH level (1.30±0.09 vs. 4.37± 0.03 µmol/g tissue). Modafinil given to rotenone-treated mice increased GSH by 130.8%, 179.2% and 161.5%, respectively, compared with the rotenone control (3.00 ± 0.07, 3.63 ± 0.12 and 3.40 ± 0.08 vs. 1.30 ±0.09 µmol/g tissue). L-dopa alone or in combination with modafinil increased GSH by 101.5% and 190.0%, respectively, compared with the rotenone control value (2.62±0.06 and 3.77±0.04 vs. 1.30 ±0.09 µmol/g tissue) (Fig.1).

**Nitric Oxide**

Mice treated with rotenone alone exhibited 106.62% increment in striatal nitric oxide content compared with the vehicle control value (76.7 ±1.3 vs. 37.1 ± 0.16 µmol/g tissue). Nitric oxide was reduced by 30.53%, 28.19% 39.19% following treatment with modafinil at 0.1, 0.2 and 0.3 mg/kg, respectively (53.2 ±1.7, 55.0 ± 1.8, 46.6 ± 1.73 vs. 76.7 ±1.3 µmol/g tissue). It decreased by 33.79% and 39.80% after treatment with L-dopa and L-dopa /modafinil combination, respectively (50.7 ± 1.1 and 46.1 ±1.28 vs. 76.7 ±1.3 µmol/g tissue) compared with rotenone group (Fig.1).

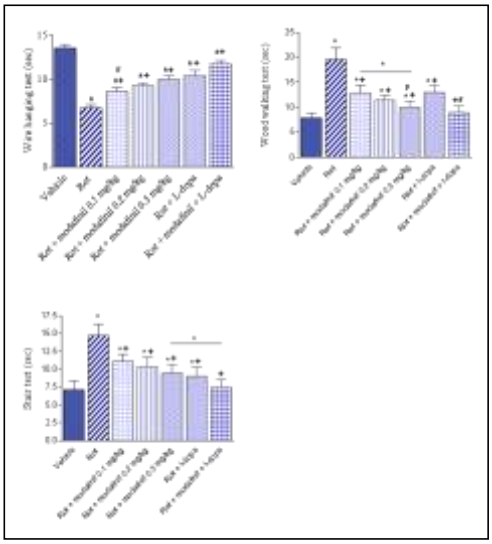

**Figure 1: Effect of modafinil treatment on striatal malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione (GSH) in rotenone-treated mice.**
**Results are means ± S.E.**
***: Significantly difference from vehicle as p< 0.05**
**+: Significantly difference from rotenone group as p<0.05**

**Dopamine level**

Rotenone administration induced significant reduction of DA content in the mice striatum by 65.99% in comparison to the vehicle- treated group (18.43±0.85 vs. 54.2± 0.78 ng/mg protein). Treatment with modafinil at doses of 0.1, 0.2, 0.3 mg/kg, markedly elevated DA content by 50.63%,63.11%,121.16%, respectively (27.77±1.27 , 30.06± 0.95 and 40.77± 3.11 vs. 18.43±0.85 ng/mg protein) as compared with rotenone group. On the other hand, administration of L-dopa or modafinil (0.2 mg/kg) combined with L-dopa caused significant elevation to DA content by 152.80%,166.19%, respectively, relative to the rotenone only-treated mice (46.6± 1.05 and 49.07± 1.05 vs. 18.43±0.85 ng/mg protein) (Fig.2) .

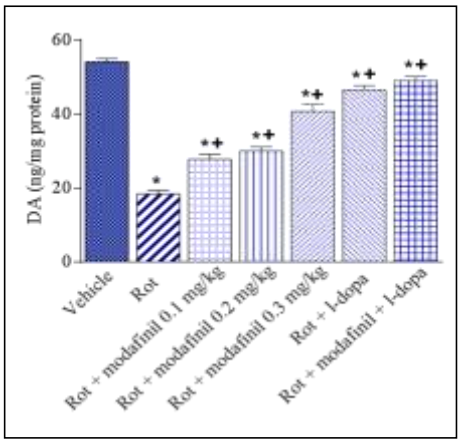


**Figure 2: Effect of modafinil treatment on striatal dopamine (DA) in rotenone-treated mice.**
**Results are means ± S.E.**
***: Significantly difference from vehicle as p< 0.05**
**+: Significantly difference from rotenone group as p<0.05**

**Brain cytokines:**

**Tumour necrosis factor-alpha**

There was significant elevation in striatal TNF-α by 480.19% in rotenone-treated group compared with the vehicle control (82.97±3.94 vs. 14.3±0.37 pg/mg protein). Modafinil given at 0.1, 0.2 and 0.3 markedly decreased brain TNF-α by 53.2%, 59.3% and 71.9% (38.83±0.47, 33.77±1.54, 23.27±1.94 vs. 82.97±3.94 pg/mg protein) compared with rotenone group. L-dopa treatment decreased TNF-α by 52.3% (39.57±3.11 vs. 82.97±3.94 pg/mg protein). Mice that received modafinil with l-dopa treatment revealed the most marked decrease in TNF-α by 75.85% (20.03±0.46 vs. 82.97±3.94 pg/mg protein) (Fig. 3).

**Interleukin-1beta**

There was significant elevation in brain IL-1β by 272.47% in rotenone-treated group compared with the vehicle control values and (148.8±3.92 vs. 39.95±2.92 pg/mg protein). Mice that received modafinil with l-dopa treatment revealed the most marked decrease in IL-1β (68.21%, 47.3±0.74 Vs 148.8±3.92 pg/mg protein) compared with rotenone group. While 0.1, 0.2 and 0.3 modafinil doses and l-dopa treatment markedly decreased brain IL-1β versus rotenone-treated group (80.27±1.79, 73.3±1.89, 53.03±3.50 and 57.87±3.75 Vs 148.8±3.92 pg/mg protein, respectively) (Fig. 3).

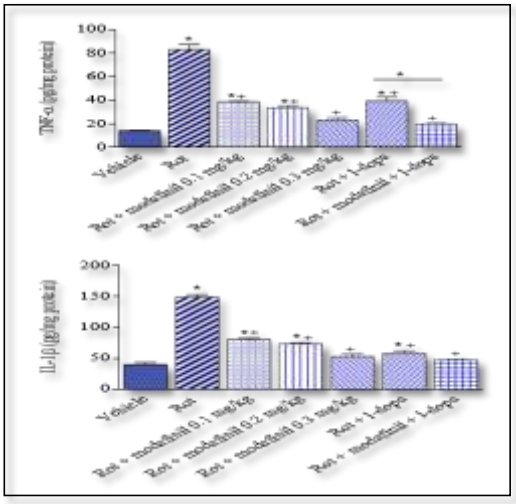

**Figure 3: Effect of modafinil treatment on striatal TNF-α and IL-1β in rotenone-treated mice.**
**Results are means ± S.E.**
***: Significantly difference from vehicle as p< 0.05**
**+: Significantly difference from rotenone group as p<0.05**

**Neurobehavioral measures**

**Wire hanging test**

**The time taken by mice to hang suspended from a steel rod was markedly decreased by rotenone compared to the vehicle-treated control groups by 50.26% (6.75±0.34 vs. 13.57±0.34).This decreasingwas enhanced by the administration of 0.1 mg/kg ,0.2 mg/kg and 0.3 mg/kg of modafinil, L-dopa or modafinil/L-dopa combination as the time taken by mice to hang suspended from a steel rod increased by (27.78 % , 37.57%, 48.15%, 54.49% and 74.07% ,respectively) (8.63±0.39, 9.29±0.26, 10±0.40, 10.43±0.57 and 11.75±0.39 vs. 6.75±0.34 sec ,respectively) compared to the rotenone-treated value. The administration of 0.1 mg/kg of modafinil compared to L-dopa-treated mice , however, resulted in increasing of the rotenone-induced impairment in motor strength by decreasing time taken by mice to hang suspended from a steel rod by (17.2 %, 8.63±0.39 vs. 10.43±0.57 sec) (Fig. 4) .**

**Wood walking test**

**The time mice spent to traverse a wooden stick was increased by rotenone compared to the vehicle-treated control by 149.2 % , (19.63±0.77 vs. 7.88±0.33 sec) , however, higher dose of modafinil treatment compared to the L-dopa treated groups decreased this time by -23.08 %, (10±0.43 vs. 13±0.47 sec).The administration of 0.1, 0.2, 0.3 mg/kg modafinil, L-dopa or modafinil/L-dopa combination decreased the time to traverse the wooden stick by 34.39 %, 41.4%, 49.04% , 33.76% and 54.14 % ,respectively, compared with the rotenone only-treated group (12.88±0.51, 11.5±0.31, 10±0.43, 13±0.47 and 9±0.43 vs.19.63±0.77,respectively) (Fig. 4).**

**Stair test**

**The time spent to ascend a stair by mice significantly increased by rotenone by 107.02 %, (14.75±0.52 vs. 7.125±0.41 sec) compared to the vehicle-treated control group. However, administration of 0.1, 0.2, 0.3 mg/kg modafinil, L-dopa or modafinil/L-dopa combination decreased this increased values by (24.58%, 29.66%, 35.59%, 38.98% and 49.15, respectively) compared to rotenone treated group; (11.125±0.33, 10.375±0.43, 9.5±0.39, 9±0.43 and 7.5±0.35 vs. 14.75±0.52 sec, respectively)(Fig. 4).**

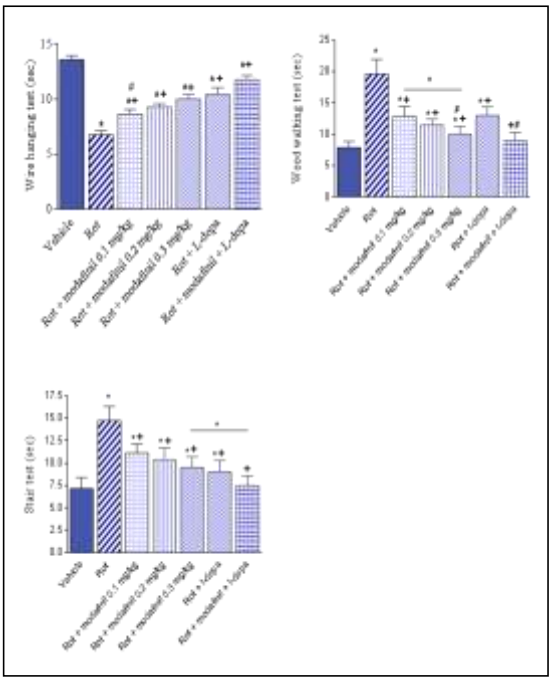

**Figure 4: Effect of modafinil treatment on behavioral dysfunction in rotenone-treated mice.**
**Results are means ± S.E.**
***: Significantly difference from vehicle as p< 0.05**
**+: Significantly difference from rotenone group as p<0.05**
**#: Significantly difference from rotenone + L- dopa group as p<0.05.**

**Histopathological results**

**Histopathological evaluation of the routine H&E slide from the negative control group showed healthy viable neurons in the substanianigra. The group receiving rotenone showed wide neuronal damage and neuronal loss with degenerated residual neurons. The L dopa group showed preservation of neuronal integrity while groups receiving the therapeutic drug modafinil showed healthy neurons in step wise fashion with increasing drug dose as in (Fig. 5).**

**This was further demonstrated by Caspase 3 immunostaining which showed positive staining in degenerated neurons in rotenone group and negative staining in the negative control group. However, mosaic pattern was shown in groups receiving modafinil where regenerating neurons stained negative for Caspase 3 and degenerated ones stained positive for Caspase 3 as in (Fig.6).**

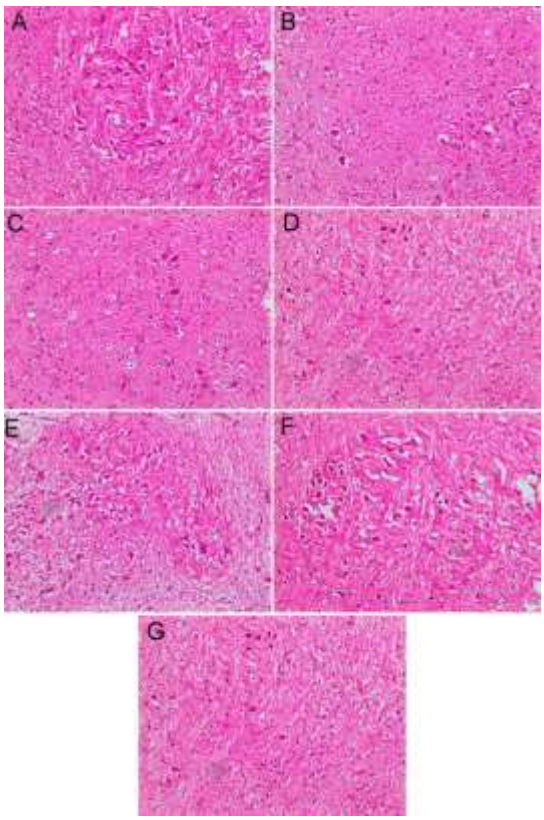


**Figure 5**: **Representative photomicrographs of Hx& E stained sections from the substantia nigra from (A) Vehicle control showing normal appearance of neurons. (B) Rotenone only showing neuronal cell loss and degeneration of residual viable neurons. (C) Rotenone + modafinil 0.1 mg/kg showing degenerated neurons with wide neuronal loss. (D) Rotenone + modafinil 0.2 mg/kg intact neurons with focal neuronal loss. (E) Rotenone + modafinil 0.3 mg/kg showing more intact neurons with few degenerated ones. (F) Rotenone + L-dopa showing intact neurons. (G) Rotenone + modafinil 0.2 mg/kg + L-dopa showing intact neurons with focal neuronal loss. Magnification x100. A&F: x200.**

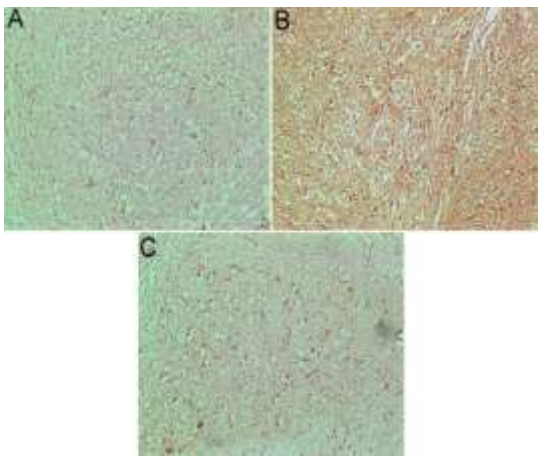


**Figure 6**: **Representative photomicrographs of caspase-3 immunoreactivity in the substantia nigra from (A) Vehicle control showing the substantia nigra lacking positive expression for caspase 3. (B) Caspase-3 over expression in the rotenone only group. (C) Rotenone + modafinil 0.2 mg/kg showing less caspase-3 expression than that found in the positive control group and areas of neuronal regeneration (x100).**

## Discussion

**The aim of this study was to investigate effect of the cerebral stimulant modafinil on striatal oxidative stress, neuroinflamation and neurodegeneration caused in the rotenone-induced experimental PD in mice. We also tested the possible modulation of the action of L-dopa when combined with modafinil. The pesticide rotenone that is widely used to induce experimental PD in laboratory animals was s.c. administrated to induce pathological, biochemical, and behavioral alterations similar to those found in Parkinson's disease. Rotenone causes neuronal cell death via increased oxidative stress [35]. The present results exhibited increased levels of oxidative stress indicated by increased lipid peroxidation, nitric oxide and decreased GSH in rotenone-treated rats compared to the control group. These findings indicating increased generation of reactive oxygen metabolites by the toxicant and are in agreement with previously published observations [36,37,38,39]. Rotenone has been shown to increase intracellular reactive oxygen metabolites [40] that attack on membrane lipids and causes lipid peroxidation and membrane alterations [41]. The rotenone-induced decrease in the level of reduced glutathione, an antioxidant and a free radical scavenger [42], is possibly due to its consumption by the increased free radicals [40]. Meanwhile, Nitric oxide increase is caused either by over-expression of nitric oxide synthases (NOSs) or by other mechanisms including glutamate excitotoxicity. Multiple lines of evidence indicate that NO reacts with superoxide anion formed during dopamine metabolism thus generating peroxynitrite that is considered one of the main damaging molecules in dopaminergic neuronal cells [43].**

**As regards to treatment with modafinil alone or combined with L.dopa showed powerful antioxidant effect manifested in reduced lipid peroxides and nitric oxide levels besides normalized GSH level; hence the neuroprotective effect presented by modafinil in this model could be through the restoration of the antioxidant pool of the brain tissue, thus preventing neuronal injury. It was shown that modafinil could reduce free-radical levels by directly acting on enzymes in the brain's free-radical scavenging system (e.g., glutathione peroxidase or superoxide dismutase) [44,45] and by reduction in adenosine resulting from reduced reactive oxygen species concentrations [46]. Also it has been shown that modafinil does have an antioxidant effect that appears to mediate neuroprotective actions in MPTP-induced neurodegeneration [47].**

**In PD, the results of neurodegenerationmay occur from not only increased levels of oxidative stress but also increased neuroinflammation [48,49]. Our data also reported an increased expression of the brain pro-inflammatory factors (i.e., (TNF-α, IL-1β), as well as**

decreased of dopamine in the striatum and loss of dopaminergic neurons in the substantia nigra, indicating degeneration in the nigrostriatal pathway compared to the control group, which supports the lines of the evidences in several previous studies of an inflammatory process in neurodegeneration caused by rotenone that have shown the pro-inflammatory cytokines TNF-alpha as well as IL-1beta are elevated in the SNc of patients with PD [50, 51,52]. These potent pro-inflammatory cytokines have with important functions in immunity, inflammation, differentiation, control of cell proliferation, and apoptosis; they can be synthesized in the central nervous system by microglia, astrocytes, and some populations of neurons [53]. TNF-α also contribute to dopaminergic neuronal death following nigrostriatal neurotoxins, such as 6-hydroxydopamine, [54] and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. [55]. Results in the present study indicate decreased levels of striatal TNF-α and IL-1β of rotenone-treated rats by modafinil administration alone or its co-administration with L. dopa. This suggests that the neuroinflammatory response elicited by the systemic administration of rotenone is inhibited by modafinil and/or L-dopa. Other researchers found that modafinil suppressed the mRNA encoding for neuro-inflammatory cytokines, TNF-α, IL-1β, followed by MPTP which support possible neurorestorative properties of modafinil [56].

Administration of rotenone in rats causes striatal dopaminergic depletion that is found in humans with PD and induces PD-like motor signs such as hypokinesia, flexed posture, rigidity and decreased locomotion [57,58,59]. The present study found that in the tests of wire hanging, wood walk, and stair climbing the administration of rotenone s.c are also indicative of dopamine depletion, impairment of motor deficits and muscle strength expressed. These data were in accordance with other studies using rotenone as an experimental PD model [60,61,62]. These results that indicating degeneration in the nigrostriatal pathway are in agreement with previous study which indicating that treatment with rotenone is capable of causing depletion of dopamine in the posterior striatum (CPu) and prefrontal cortex , reducing immunoreactivity in CPu and induction of parkinsonian behavioural symptoms [63].

In this study, the higher dose of modafinil and the combination of L-dopa with modafinil were effective in ameliorating the rotenone-induced motor disabilities. Mignot et al. [18] found that that modafinil might act by blocking dopamine transporter, and they pointed out that modafinil has more potent behavioral effects than some molecules that bind with a much greater affinity to the dopamine reuptake transporter. Other study suggested that modafinil acutely improved dopamine levels and blocked dopamine transporters in the human brain [64]. Modafinil enhanced the recovery of dopaminergic system, as verified by increased mRNA encoding TH, striatal DA concentration, [56], and following MPTP –induced PD [65,66].

The improvement that noticed by using modafinil and L-dopa/modafinil suggested that PD patients might benefit from these treatments,many PD patients have EDS and excessive daytime sleepiness [67,68,69]. Modanifil was reported to increase concentrations of DA, NA and 5HT which may have impact on increasing wakefulness and also be likely to impose the good effect on motor symptoms of PD patients [56]. previous study confirm the findings of using modafinil in MPTP-treated monkeys [70], since, modafinil has protective properties against MPTP damage of the substantia nigra neurons which is reflected on functional outcome, as seen in clinical and abnormal involuntary movement scores and behavioral tests concerning coordination andmovements, and on monoamine levels in the striatum [71].

Histological, theneuronal cell loss and degeneration of residual viable neurons in the substanianigra in mice that only was given rotenone. These findings in accordance with previous results which indicating nigrostriatal cell loss after systemic rotenone administration in rodents [57,58,60,63] and further strengthen the notion involving pesticidesin rural areas the development of Parkinson's disease [72]. However, the more intact neurons with few degenerated ones were present in mice given high dose of modafinil only and also that given modafinil combined with L.dopa. Apoptosis induced by rotenone that was confirmed by the increased cleaved caspase-3 expression in dopaminergic neurons [58,73,74,75].This effect was noticed in mice that only given rotenone in this study as a strong cleaved caspase-3 expression could be detected in degenerated neurons after rotenone injection. Caspase proteins are cysteine proteases and members of the interleukin-1β-converting enzyme family involved in the initiation and execution of programmed cell death or apoptosis [76,77]. In the study apoptosis decreased by 0.2 mg/kg modafinil that resulted in very few caspase-3 positive cells being present, suggesting that the drug inhibits rotenone-induced brain cell damage possibly through interference with caspase-3 activation. These results may be of significance in the use of modafinil in PD [78].

## Conclusion

This study has attempted to highlight the effects of the modafinil drug in PD, which has several anti-parkinsonian effects and may decrease dopaminergic cell loss through inhibition of oxidative stress, neuroinflammation, and apoptosis. From these findings, we suggest modafinil adjuvant therapy as a new approach in PD treatment.

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