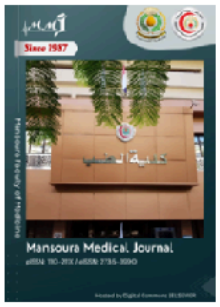
MANSOURA MEDICAL JOURNAL

ISSN - Print: 1110-211X - Online: 2735-3990

journal homepage: mmj.mans.edu.eg



Volume 52 | Issue 2 Article 3

Potential Neuroprotective Effect of Artemisinin in a Rotenone-induced Mice Model of Parkinson's Disease

Nada M. Hamada

Clinical Pharmacology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt,

nadamohammed88@mans.edu.eg

Hanan Eissa

Clinical Pharmacology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Nirmeen Megahed

Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Mohamed H. Daba

Clinical Pharmacology Department, Faculty of Medicine, Damietta University, Damietta, Egypt

Ahlam Elmasry

Clinical Pharmacology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

ORIGINAL STUDY

Potential Neuroprotective Effect of Artemisinin in a Rotenone-Induced Mice Model of Parkinson's Disease

Nada M. Hamada, MSc, PhD [a,*], Hanan Eissa [a,d], Nirmeen Megahed [b], Mohamed H. Daba [c], Ahlam Elmasry [a]

[a] Department of Clinical Pharmacology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[b] Department of Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[c] Department of Clinical Pharmacology, Faculty of Medicine, Damietta University, Damietta, Egypt
[d] Department of Pharmacy Practice, Delta University for Science and Technology, Gamasa, Egypt

Abstract

Background: **Parkinson's disease (PD) is characterized by motor dysfunctions, including tremors, rigidity, and bradykinesia. Medications for PD alleviate motor symptoms rather than targeting disease pathogenesis. Recently discovered features of artemisinin suggested that it might be used to treat neurodegenerative diseases.**

Objective: **To explore the possible neuroprotective effect of artemisinin in different doses in a rotenone-induced model of PD.**

Materials and methods: **A total of 25 mice were randomly divided into five groups: group 1, negative control; group 2, positive control; and groups 3, 4, and 5, artemisinin treated (30, 40, and 50 mg/kg, respectively). For PD induction, rotenone (3 mg/kg/day) was administered intraperitoneally for 42 days (6 weeks). Behavioral assessment (open field and parallel rod tests) was performed twice: after 3 weeks of induction and at the end of the study (after 6 weeks). After scarification, an immunohistochemical analysis of tyrosine hydroxylase in the substantia nigra pars compacta was conducted.**

Results: **Artemisinin administration at a dose of 50 mg/kg produced more pronounced significant protective effects compared with other diseased and treated groups as guided by behavioral assessment tests and immunostaining analysis.**

Conclusions: **Artemisinin showed a promising protective effect in the rotenone-induced PD model. Further research studies are needed to validate this effect.**

Keywords: Artemisinin, Parkinson's disease, Substantia nigra, Tyrosine hydroxylase

1. Introduction

Parkinson's disease (PD) is considered the second most prevalent neurodegenerative disease after Alzheimer's disease (AD). Approximately 1–3% of people older than 60 years are affected (Tysnes and Storstein, 2017). It is characterized by a substantial loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc). It presents primarily as motor impairments, including tremors, rigidity, and bradykinesia. The pathogenesis of PD is multifactorial with several implicated mechanisms such as apoptosis, neuroinflammation, and oxidative stress (Cheng et al., 2021). At present, PD is mainly treated with drugs that primarily attenuate motor manifestations, such as cholinergic antagonists, DA agonists, and L-dopa. These medications aim to raise the DA levels in the striatum to alleviate the associated motor impairment of PD. However, they do not offer a long-term solution, because they all become less effective as DA neuron damage

Received 1 February 2023; revised 22 February 2023; accepted 24 February 2023.
Available online 20 June 2023

* Corresponding author at: Department of Clinical Pharmacology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt.
E-mail address: nadamohammed88@mans.edu.eg (N.M. Hamada).

https://doi.org/10.58775/2735-3990.1374

worsens (Wang et al., 2020). Prolonged use of these medications has numerous hazardous adverse effects (Ahmad et al., 2022). Consequently, recognizing effective, cheap, and nontoxic agents that can attenuate loss of DA neurons would make significant progress in the management of PD.

Among the numerous models for PD, the use of a rotenone-induced PD model received great attention for two reasons: (a) it replicates most of the motor deficits and the histopathological features of PD, such as LB, and (b) rotenone and other pesticides are potent mitochondrial respiration inhibitors that are associated with a high incidence of sporadic PD among the population of the rural areas (Xiong et al., 2013). Rotenone is a naturally occurring toxin that readily crosses the BBB as it is a lipophilic compound (Thirugnanam & Santhakumar, 2022). Rotenone can induce substantia nigra DA neuron loss and aggregation of α-synuclein, which are considered hallmarks of PD neuropathology (Innos and Hickey, 2021). Thus, a rotenone PD model was chosen to reproduce behavioral, biochemical, and pathological features of PD in this study.

Artemisinin is a well-known antimalarial drug that has saved millions of lives worldwide. No major adverse effects were reported following the long-term use of artemisinin (Lu et al., 2019). Recently observed neuroprotective properties of artemisinin have not yet been fully investigated. Artemisinin was found to greatly enhance cognitive performance and neuronal functions in AD transgenic mice. These effects were mediated by decreasing oxidative stress, the release of pro-inflammatory mediators, and apoptosis-related proteins (Zhao et al., 2020). Artemisinin has shown neurotrophic and neuroprotective effects in pheochromocytoma (PC12) cells (Zeng et al., 2017; Zheng et al., 2016). In an in vitro model of PD, artemisinin protected dopaminergic neurons against 1-methyl-4-phenyliodine iodide-induced damage by the antiapoptotic and antioxidant activities and inhibition of autophagy (Yan et al., 2021). All these properties of artemisinin and their obvious association with the pathogenic mechanisms of PD led us to question its potential use as a neuroprotective agent in different doses in a rotenone-induced model of PD.

2. Material and methods

2.1. Drugs and chemicals

Rotenone was obtained from Abcam, Cambridge, UK (code: ab143145), in the form of raw material powder and dissolved in 0.5% carboxymethylcellulose solution. Artemisinin (purity≥98%) was obtained from Nanjing Fuxing Biological Technology Co. Ltd, Jiangsu, China (cat. no.RL04438), in the form of powder and dissolved in 0.5% carboxymethylcellulose solution.

2.2. Sample size

We calculated the sample size using the resource equation method (Arifin and Zahiruddin, 2017):

$$n = \mathrm{DF}/k + 1.$$

where DF = the error degrees of freedom, k = the number of mice groups, and n = the number of mice per group.

The maximum allowed DF is 20, and k is 5. Thus, $n = 20/5 + 1 = 5$ mice per group.

2.3. Animals

A total of 25 male C57BL/6 mice (20–25 g) were purchased from Mansoura Experimental Research Center (MERC). Mice were maintained for 1 week under standard laboratory conditions to acclimate before starting the experiment, at room temperature, under a controlled 12 h light/dark cycle. Mice had unrestricted access to food and water all over the days of the experiment. The local animal ethics committee approved this experiment design.

2.4. Experimental design

Mice were randomly divided into five groups ($n = 5$ per group):

Group 1 was the negative control group, which received 0.5% carboxymethylcellulose, via intraperitoneal injection daily for 42 days.

Group 2 was the rotenone-induced PD group (positive control group), which received rotenone (3 mg/kg/day), dissolved in 0.5% carboxymethylcellulose, injected intraperitoneally for 42 days (Salama et al., 2017).

Group 3 was rotenone-induced PD group treated with artemisinin (30 mg/kg/day) via intraperitoneal injection once daily for 42 days, 1 h before rotenone.

Group 4 was rotenone-induced PD group treated with artemisinin (40 mg/kg/day) as previously described (Shi et al., 2013).

Group 5 was rotenone-induced PD group treated with artemisinin (50 mg/kg/day) as previously described.

2.5. Assessment

2.5.1. Behavioral tests

Mice were behaviorally assessed after 3 and 6 weeks.

2.5.2. Open field test

This test assessed locomotor activity. The open field was conducted with the use of a square wooden box (40 cm × 40 cm) and a wall (35 cm high) divided into 16 subsquares. Before assessment, mice were trained for the open-field test, and after that, they were observed for 5 min. The following parameters were objectively evaluated using the ANY MAZE video tracking system (Stoelting Co., Wood Dale, Illinois, USA): distance traveled by the mice, average speed, total immobility time, and total number of immobility episodes were recorded.

2.5.3. Parallel rod test

Motor coordination and balance were evaluated for mice using a parallel rod floor apparatus (20 × 20 × 30 cm clear acrylic box with a removable top). Mice were adapted to the parallel rod test before assessment and then observed for 5 min. The number of foot slip errors was calculated for all mice.

2.5.4. Immunohistochemistry

At the end of the experiment, mice were killed. Dissected brains were washed with ice-cold saline. Each brain hemisphere was paraffin-embedded after being preserved with 4% paraformaldehyde in 0.1 M phosphate buffer overnight. All paraffinized tissues were removed at the level of the substantia nigra, dissected to a thickness of 4 mm, and then allowed to air dry for an entire night at room temperature. The sections were then deparaffinized, rehydrated, and available for tyrosine hydroxylase (TH) immunohistochemical staining. Brain sections were incubated with a rabbit polyclonal anti-TH antibody (diluted 1: 100; cat. no. YPA2228; Chongqing Biospes Co., China) overnight at 4 °C. A biotinylated secondary antibody (diluted 1: 10 000; Sigma-Aldrich, Saint Louis, Missouri, USA) was used, followed by the avidin–biotin–peroxidase complex at room temperature for 10 min. Following the addition of diaminobenzidine, the labeling was identified. Photographing was done by using an Olympus microscope. The histological examination was blindly done for counting the TH-immunopositive cells. VideoTest Morphology software (VideoTesT, Ltd., St.Petersburg, Russia) was used for image analysis with a specific built-in routine for particle analysis and counting.

2.6. Statistical analysis

Results were statistically analyzed using SPSS, version 26 (International Business Machines co., New York, USA). The Shapiro–Wilk test was used to evaluate normality. Results were reported as mean ± SD. Analysis of variance test was performed followed by a post-hoc Tukey test for comparison between groups. Paired *t* test was done to compare between results at 3 and 6 weeks. The *P* value is considered significant when it equals 0.05 or less.

3. Results

3.1. Behavioral tests

3.1.1. Open field test

After 3 weeks: after 3 weeks, the rotenone-induced PD group exhibited a significant decrease in the total distance traveled (1.72 ± 0.05) as compared with the control group (3.74 ± 0.06) ($P < 0.001$). Administration of artemisinin at both doses of 30 and 40 mg/kg produced a nonsignificant improvement in the total distance traveled (1.73 ± 0.05 and 1.75 ± 0.04, respectively) in comparison with the rotenone-induced PD model ($P > 0.05$). However, the artemisinin-treated group at a dose of 50 mg/kg produced a significant improvement in the total distance traveled (2.14 ± 0.1) in comparison with the rotenone-induced PD model and other artemisinin-treated groups ($P < 0.001$) (Fig. 1a).

The rotenone-induced PD group exhibited a significant decrease in the average speed (0.046 ± 0.011) as compared with the control group (0.11 ± 0.01) ($P < 0.001$). Administration of artemisinin at both doses of 30 and 40 mg/kg produced a nonsignificant improvement in the average speed (0.05 ± 0.007 and 0.054 ± 0.011, respectively) in comparison with the rotenone-induced PD model ($P > 0.05$). The artemisinin-treated group at a dose of 50 mg/kg produced a significant improvement in the average speed (0.068 ± 0.008) compared with the rotenone-induced PD model ($P < 0.05$) and a nonsignificant improvement in the average speed in comparison with other artemisinin-treated groups ($P > 0.05$) (Fig. 1b).

On the contrary, the rotenone-induced PD group exhibited a significant increase in the total immobility time (25.56 ± 1.28) as compared with the control group (5.79 ± 0.23) ($P < 0.001$). Administration of artemisinin at a dose of 30 mg/kg produced a nonsignificant decrease in the total immobility time (24.68 ± 0.76) in comparison with the rotenone-induced PD model ($P > 0.05$). Administration of artemisinin at a dose of 40 mg/kg produced a

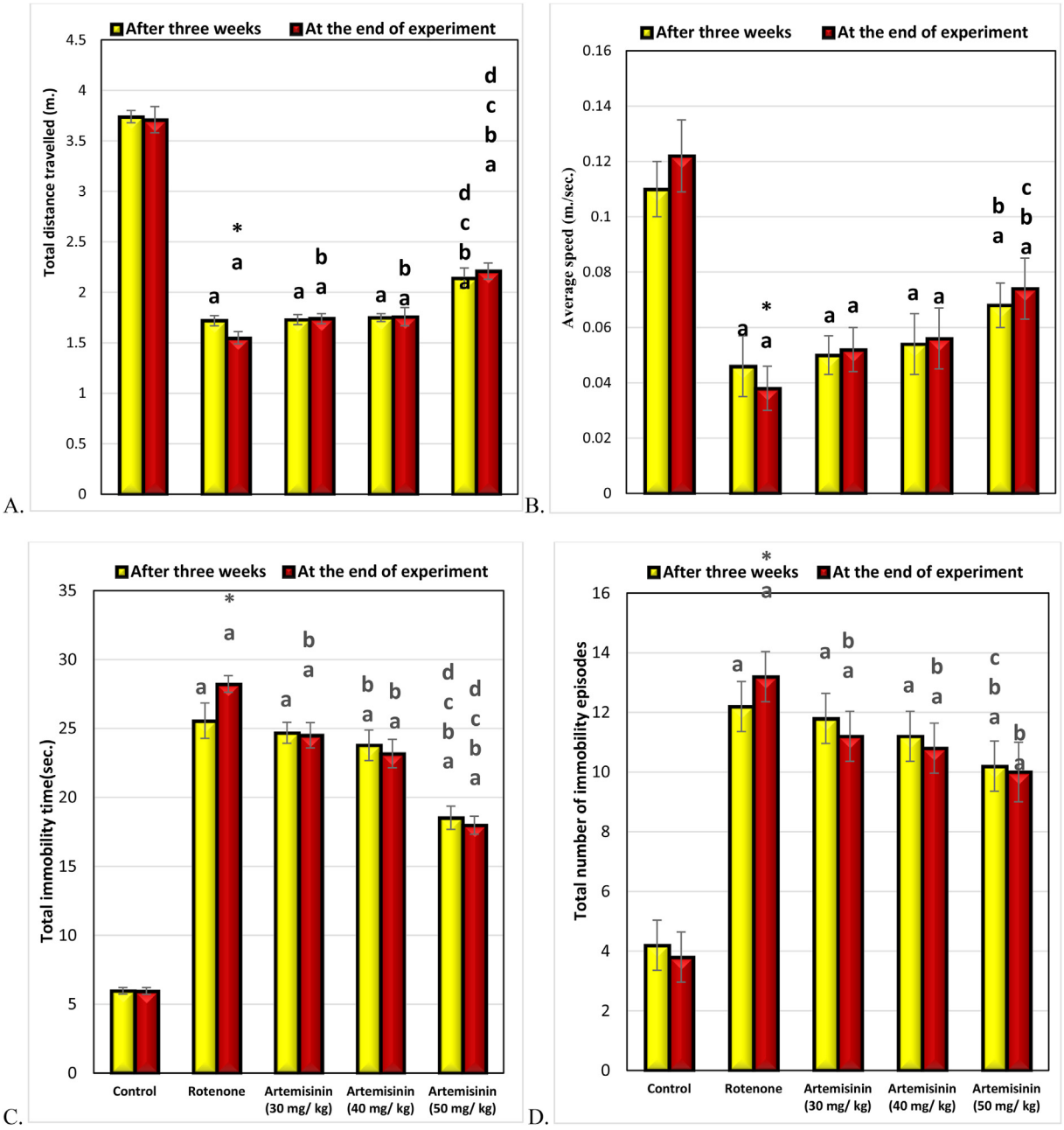


*Fig. 1. Effect of different doses of artemisinin on locomotion in open field test after 3 weeks and at the end of the experiment: analysis by ANOVA followed by Tukey post-hoc test ($P \leq 0.05$, $n = 5$ mice). A paired t test to compare two time points for the same group: a: versus control, b: versus rotenone-induced PD group, c: versus artemisinin (30 mg/kg) group, d: versus artemisinin (30 mg/kg) group, *: versus the same group after 3 weeks. ANOVA, analysis of variance; PD, Parkinson's disease.*

significant decrease in the total immobility time (23.78 ± 1.11) in comparison with the rotenone-induced PD model ($P < 0.05$). The artemisinin-treated group at a dose of 50 mg/kg produced a significant decrease in the total immobility time (18.52 ± 0.84) in comparison with the rotenone-induced PD model and other artemisinin-treated groups ($P < 0.001$) (Fig. 1c).

The rotenone-induced PD group exhibited a significant increase in the total number of immobility episodes (12.2 ± 0.84) as compared with the control group (4.2 ± 0.84) ($P < 0.001$). Administration of artemisinin at doses of 30 and 40 mg/kg produced a nonsignificant decrease in the total number of immobility episodes (11.8 ± 0.84 and 11.2 ± 0.84, respectively) in comparison with the rotenone-

induced PD model ($P > 0.05$). The artemisinin-treated group at a dose of 50 mg/kg produced a significant decrease in the total number of immobility episodes (10.2 ± 0.84) in comparison with the rotenone-induced PD model ($P < 0.01$) and artemisinin-treated group at a dose of 30 mg/kg ($P < 0.05$) (Fig. 1d).

After 6 weeks: after 6 weeks, the rotenone-induced PD group exhibited a significant decrease in the total distance traveled (1.55 ± 0.06) as compared with the control group (3.71 ± 0.13) ($P < 0.001$). Administration of artemisinin at different doses (30, 40, and 50 mg/kg) produced a significant improvement in the total distance traveled (1.74 ± 0.05, 1.76 ± 0.09, and 2.21 ± 0.08, respectively) in comparison with the rotenone-induced PD model ($P < 0.05$, 0.01, and 0.001, respectively). The artemisinin-treated group at a dose of 50 mg/kg produced a significant improvement in the total distance traveled in comparison with other artemisinin-treated groups ($P < 0.001$) (Fig. 1a).

The rotenone-induced PD group exhibited a significant decrease in the average speed (0.038 ± 0.008) as compared with the control group (0.122 ± 0.013) ($P < 0.001$). Administration of artemisinin at both doses of 30 and 40 mg/kg produced a nonsignificant improvement in the average speed (0.052 ± 0.008 and 0.056 ± 0.011, respectively) in comparison with the rotenone-induced PD model ($P > 0.05$). The artemisinin-treated group at a dose of 50 mg/kg produced a significant improvement in the average speed (0.074 ± 0.011) in comparison with the rotenone-induced PD model ($P < 0.001$) and artemisinin-treated group at a dose of 30 mg/kg ($P < 0.05$) (Fig. 1b).

On the contrary, the rotenone-induced PD group exhibited a significant increase in the total immobility time (28.22 ± 0.6) as compared with the control group (5.95 ± 0.25) ($P < 0.001$). Artemisinin-treated groups at doses of 30 and 40 mg/kg produced a significant decrease in the total immobility time (24.5 ± 0.92 and 23.17 ± 1.03, respectively) in comparison with the rotenone-induced PD model ($P < 0.001$). The artemisinin-treated group at a dose of 50 mg/kg produced a significant decrease in the total immobility time (17.98 ± 0.65) in comparison with the rotenone-induced PD model and other artemisinin-treated groups ($P < 0.001$) (Fig. 1c).

The rotenone-induced PD group exhibited a significant increase in the total number of immobility episodes (13.2 ± 0.84) as compared with the control group (3.8 ± 0.84) ($P < 0.001$). Administration of artemisinin at both doses of 30 and 40 mg/kg produced a significant decrease in the total number of immobility episodes (11.2 ± 0.84 and 10.8 ± 0.84, respectively) in comparison with the rotenone-induced PD model ($P < 0.05$ and 0.01, respectively). The artemisinin-treated group at a dose of 50 mg/kg produced a significant decrease in the total number of immobility episodes (10 ± 1) in comparison with the rotenone-induced PD model ($P < 0.001$) and a nonsignificant decrease in comparison with other artemisinin-treated groups ($P > 0.05$) (Fig. 1d).

3.1.2. Parallel rod test

After 3 weeks: after 3 weeks, the rotenone-induced PD group exhibited a significant increase in the total number of foot slip errors (15.4 ± 1.14) as compared with the control group (4.2 ± 0.84) ($P < 0.001$). Administration of artemisinin at both doses of 30 and 40 mg/kg produced a nonsignificant decrease in the total number of foot slip errors (15.2 ± 0.84 and 15 ± 1, respectively) in comparison with the rotenone-induced PD model ($P > 0.05$). The artemisinin-treated group at a dose of 50 mg/kg produced a significant decrease in the total number of foot slip errors (13.2 ± 0.84) in comparison with the rotenone-induced PD model and other artemisinin-treated groups ($P < 0.05$) (Fig. 2).

After 6 weeks: after 6 weeks, the rotenone-induced PD group exhibited a significant increase in the total number of foot slip errors (16.2 ± 0.84) as compared with the control group (3.8 ± 0.84) ($P < 0.001$). Administration of artemisinin at both doses of 30 and 40 mg/kg produced a nonsignificant decrease in the total number of foot slip errors (15 ± 1 and 14.8 ± 0.84, respectively) in comparison with the rotenone-induced PD model ($P > 0.05$). The artemisinin-treated group at a dose of 50 mg/kg produced a significant decrease in the total number of foot slip errors (12.8 ± 0.84) in comparison with the rotenone-induced PD model ($P < 0.001$) and artemisinin-treated groups at both doses of 30 and 40 mg/kg ($P < 0.01$ and 0.05, respectively) (Fig. 2).

According to the results of behavioral tests, the rotenone-induced PD group exhibited a significant decrease in the total distance traveled and the average speed after 6 weeks in comparison with those after 3 weeks ($P < 0.05$). In addition, the rotenone-induced PD group exhibited a significant increase in the total immobility time, the total number of immobile episodes, and foot slip errors after 6 weeks in comparison with those after 3 weeks ($P < 0.05$). Artemisinin-treated groups at different doses showed nonsignificant differences in all assessed parameters after 6 weeks for each group in comparison with those after 3 weeks ($P > 0.05$).

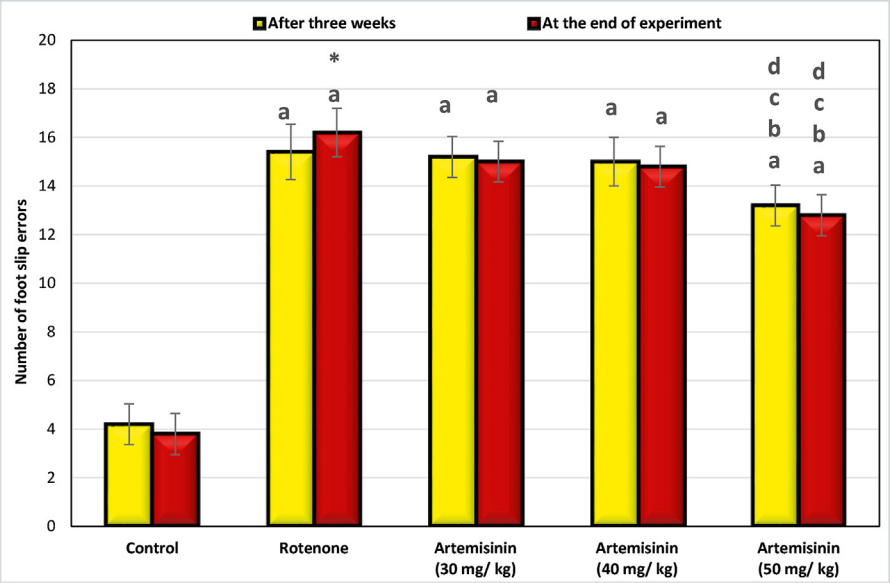


*Fig. 2. Effect of different doses of artemisinin on motor coordination in parallel rod test after 3 weeks and at the end of the experiment: analysis by ANOVA followed by Tukey post-hoc test ($P \leq 0.05$, $n = 5$ mice). A paired t test to compare two time points for the same group: a: versus control, b: versus rotenone-induced PD group, c: versus artemisinin (30 mg/kg) group, d: versus artemisinin (30 mg/kg) group, *: versus the same group after 3 weeks. ANOVA, analysis of variance; PD, Parkinson's disease.*

3.2. Immunohistochemistry

The rotenone-induced PD group showed a significantly lower percentage of TH-positive neurons in comparison with the control group (expressed as 100%) ($P < 0.001$). However, the administration of artemisinin at a dose of 30 mg/kg produced a nonsignificant higher percentage of TH-positive neurons in comparison with the rotenone-induced PD group ($P > 0.05$). The artemisinin-treated group at a dose of 40 mg/kg showed a significantly higher percentage of TH-positive neurons in comparison with the rotenone-induced PD group ($P < 0.05$). However, the artemisinin-treated group at a dose of 50 mg/kg showed a significantly higher percentage of TH-positive neurons compared with the rotenone-induced PD group ($P < 0.001$) and artemisinin-treated groups at both doses of 30 and 40 mg/kg ($P < 0.001$ and 0.05, respectively) (Table 1 and Fig. 3).

4. Discussion

The present study aimed to investigate the possible neuroprotective effect of artemisinin in different doses in a rotenone-induced model of PD. The neuroprotective effect was investigated using behavioral tests and immunohistochemistry. We studied the effect of artemisinin at two time points: after 3 weeks and at the end of the study. Artemisinin could protect the brains of mice against rotenone neurotoxicity.

In the present study, rotenone was used to induce a PD model in mice. To investigate how medications affect PD prevention or treatment, numerous animal models are used. Genetic and neurotoxic models are the two primary groups that currently exist for PD animal models. There are numerous neurotoxin-based models of PD that display a large loss of nigrostriatal DA neurons, including paraquat, rotenone, 6-hydroxydopamine, and 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (Zeng et al., 2018).

Among the numerous models for PD, a rotenone PD model was chosen to reproduce behavioral and pathological features of PD in this study for three reasons: (a) it replicates most of the clinical features of PD, such as its chronic progressive nature (Salama et al., 2017); (b) rotenone and other pesticides are potent mitochondrial respiration inhibitors and associated with the higher incidence of sporadic PD among the population of the rural areas (Xiong et al., 2013); and (c) rotenone can induce substantia nigra DA neuron loss, which is one of the hallmarks of PD neuropathology (Innos and Hickey, 2021). In the current study, intraperitoneal injection of rotenone (3 mg/kg/day) for 42 days led to a deficit in the motor activity of mice. Additionally, rotenone administration induced DA cell degeneration in the SNpc.

Behavioral tests play an important role in the research of symptom-relieving medications as they can be translated into clinical performance in patients. In the present study, the rotenone-induced

Table 1. Effect of different doses of artemisinin on the percentage of tyrosine hydroxylase-positive neurons after immunostaining of the substantia nigra pars compacta.

Mice groups	Rotenone	Artemisinin (30 mg/kg)	Artemisinin (40 mg/kg)	Artemisinin (50 mg/kg)
Percentage of TH + ve neurons (% of control)	27.72 ± 7.78	38.04 ± 6.08	45.11 ± 9.13[b]	63.59 ± 11.46[bcd]

Results were represented as mean ± SD.
Analysis by analysis of variance followed by Tukey post-hoc test ($P \leq 0.05$, $n = 5$ mice). The values were reported as a percentage compared with the control group (expressed as 100%). Superscript letters expressed significance as b: versus rotenone-induced Parkinson's disease group, c: versus artemisinin (30 mg/kg) group, d: versus artemisinin (30 mg/kg) group.

PD group showed a significantly lower speed and shorter distance traveled but longer immobility duration and more number of immobility episodes as compared with the control mice. Teema et al. (2016) reported that the rotenone group showed lower ambulation but longer immobility duration. Furthermore, the rotenone-induced PD group showed a higher number of foot slip errors in the parallel rod test compared with the control mice. In agreement, Salama et al. (2017) reported a significantly higher number of foot slips in the rotenone group (an average of six slips) in comparison with the control group (an average of one).

In the current study, behavioral tests were made at two time points to assess the progression of the PD model. The rotenone-induced PD group showed deterioration in all evaluated behavioral parameters in either open field or parallel rod tests after 6 weeks

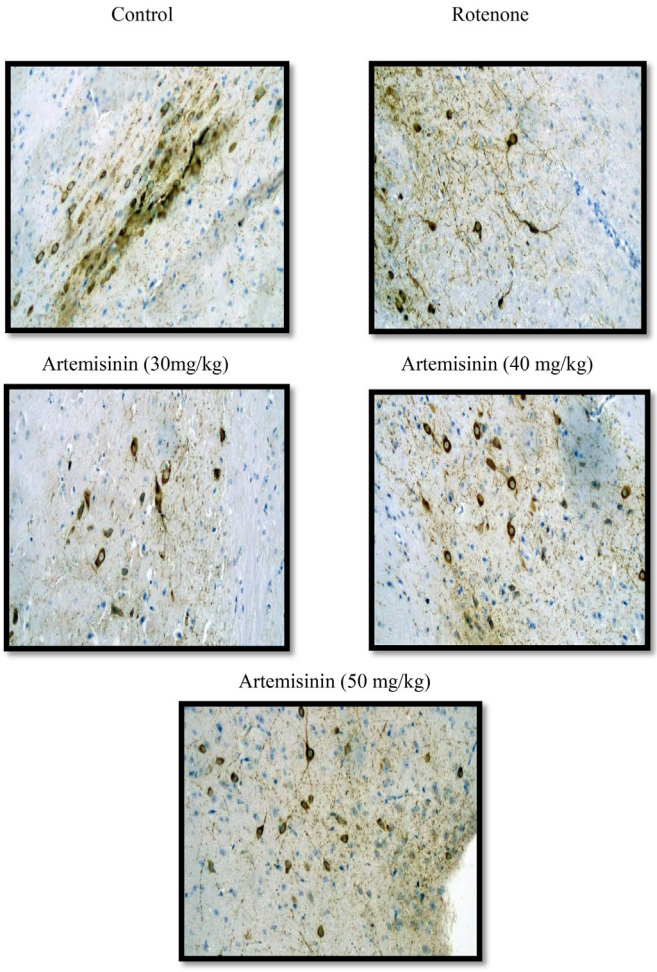


Fig. 3. Sections from the substantia nigra pars compacta neurons immunohistochemically stained for tyrosine hydroxylase (TH): showing the effect of different doses of artemisinin on the percentage of TH + ve neurons; as artemisinin at a dose of 30 mg/kg (38.04 ± 6.08), artemisinin at a dose of 40 mg/kg (45.11 ± 9.13), and artemisinin at a dose of 50 mg/kg (63.59 ± 11.46).

compared with those after 3 weeks. Salama et al. (2012) reported that locomotor disturbances induced by rotenone were potentiated with time progress.

In the present study, the artemisinin-treated group at a dose of 50 mg/kg showed significant protective effects on motor performance all over the study compared with the rotenone-induced PD group. Artemisinin-treated groups at both doses of 30 and 40 mg/kg showed significant protective effects on certain behavioral parameters (the total distance traveled, the total immobility time, and the number of immobile episodes), and nonsignificant improvement was observed during the assessment of the average speed of mice and the total number of foot slip errors compared with the rotenone-induced PD group.

According to Wang et al. (2022), it was observed that artemisinin improved neurological impairments in mice that had intracerebral hemorrhage after behavioral evaluation. This result may be due to the reduction of oxidative stress and neuroinflammation. In the AD mouse model, artemisinin greatly enhanced cognitive performance and neurological functions (Zhao et al., 2020).

In the current study, the percent of TH + ve cells in SNpc of the rotenone-induced PD group was significantly lower in comparison with the control group. According to Teema et al. (2016), rotenone administration led to a significant loss of TH-positive DA neurons in the SNpc. In some cellular and animal experimental models, rotenone was found to cause apoptotic cell death. According to Ahmadi et al. (2003), the administration of rotenone to primary DA neuronal cell culture enhanced the number of apoptotic TH-immunopositive neurons, which was associated with the upregulation of caspase-3 immunoreactivity. Chronic rotenone intoxication caused apoptotic cell death in the striata of rats, which may be attributed to increasing mitochondrial ROS (Lin et al., 2012).

The selective loss of DA neurons in the nigrostriatal pathway is the main cause of the development of PD clinical motor features and one of the pathological hallmarks of PD. The TH enzyme, which is generated in the DA neurons of the SNpc, is known to be the rate-limiting enzyme that catalyzes the initial step of DA synthesis. DA is retained in synaptic vesicles and released in the striatum in response to stimuli to perform its physiological activity (Habib et al., 2022). Histopathological assessment is used to study the improvement of pathology after medical therapy (Salama et al., 2017).

In the present study, administration of artemisinin at doses of 40 and 50 mg/kg prevented the rotenone-induced loss of DA neurons, whereas the artemisinin-treated group at a dose of 30 mg/kg exhibited nonsignificant protection against the rotenone-induced loss of DA neurons. According to Zhao et al. (2020), artemisinin can protect neuronal cells in-vivo and in-vitro AD models by activating the ERK/CREB pathway and inhibiting the apoptotic pathway, at least in part. In agreement, Yan et al. (2021) reported that artemisinin had a protective effect against oxidative stress-induced apoptosis in DA neurons by decreasing oxidative damage and autophagy. After 24-h treatment with both MPP+ and artemisinin, the viability of neuroblastoma cell line (SH-SY5Y) nerve cells was assessed, and it was observed that artemisinin greatly increased the survival of MPP + -treated nerve cells. This result was associated with its antioxidant effect, which was determined by measuring the levels of MDA, GSH, SOD, and ROS. It was also discovered that artemisinin decreased the expression of cleaved caspase-3, and thus, it slowed down the rate of cell apoptosis caused by MPP+.

Moreover, Peng et al. (2022) showed that artemisinin had a protective effect on PC12 cells against oxygen-glucose deprivation/reperfusion injury. Artemisinin was able to increase cell viability and decrease ROS production and cell apoptosis. Artemisinin attenuated PC12 cell apoptosis by activating the ERK1/2/CREB/BCL-2 pathway. Lin et al. (2018) found that the antiapoptotic effect of artemisinin may be attributed to the activation of the Akt signaling pathway. Furthermore, this effect was inhibited by MK2206, a highly selective Akt inhibitor, confirming that Akt pathway stimulation contributed to the neuroprotective action of artemisinin.

5. Conclusion

Artemisinin showed protective effects against rotenone-induced neurotoxicity in a dose-dependent manner, denoting its possible neuroprotective effect in patients with PD. Our study showed that artemisinin administration at a dose of 50 mg/kg was the most effective to mitigate changes induced by rotenone as guided by behavioral assessment and immunohistochemistry.

Conflicts of interest

The present study was supported by a funding grant supplied by Competitive Funding Projects, Postgraduate Research and Cultural Affairs Sector, Mansoura University and approved by the Institutional Research Board (IRB) – Mansoura Faculty of Medicine (code: RP.21.03.100).

References

Ahmad, J., Haider, N., Khan, M.A., Md, S., Alhakamy, N.A., Ghoneim, M.M., Cheng, X.X., et al., 2022. Novel therapeutic interventions for combating Parkinson's disease and prospects of nose-to-brain drug delivery. Biochem. Pharmacol. 195, 114849.

Ahmadi, F.A., Linseman, D.A., Grammatopoulos, T.N., Jones, S.M., Bouchard, R.J., Freed, C.R., Heidenreich, K.A., Zawada, W.M., 2003. The pesticide rotenone induces caspase-3-mediated apoptosis in ventral mesencephalic dopaminergic neurons. J. Neurochem. 87, 914–921.

Arifin, W.N., Zahiruddin, W.M., 2017. Sample size calculation in animal studies using resource equation approach. Malays. J. Med. Sci. 24, 101.

Cheng, Q., Chen, J., Guo, H., Lu, J.L., Zhou, J., Guo, X.Y., et al., 2021. Pyrroloquinoline quinone promotes mitochondrial biogenesis in rotenone-induced Parkinson's disease model via AMPK activation. Acta Pharmacol. Sin. 42, 665–678.

Habib, C.N., Mohamed, M.R., Tadros, M.G., Tolba, M.F., Menze, E.T., Masoud, S.I., 2022. The potential neuroprotective effect of diosmin in rotenone-induced model of Parkinson's disease in rats. Eur. J. Pharmacol. 914, 174573.

Innos, J., Hickey, M.A., 2021. Using rotenone to model Parkinson's disease in mice: a review of the role of pharmacokinetics. Chem. Res. Toxicol. 34, 1223–1239.

Lin, T.K., Cheng, C.H., Chen, S.D., Liou, C.W., Huang, C.R., Chuang, Y.C., et al., 2012. Mitochondrial dysfunction and oxidative stress promote apoptotic cell death in the striatum via cytochrome c/caspase-3 signaling cascade following chronic rotenone intoxication in rats. Int. J. Mol. Sci. 13, 8722–8739.

Lin, S.P., Li, W., Winters, A., Liu, R., Yang, S.H., 2018. Artemisinin prevents glutamate-induced neuronal cell death via Akt pathway activation. Front. Cell. Neurosci. 12, 1–9.

Lu, B.W., Baum, L., So, K.F., Chiu, K., Xie, L.K., 2019. More than anti-malarial agents: therapeutic potential of artemisinins in neurodegeneration. Neural Regen Res 14, 1494–1498.

Peng, T., Li, S., Liu, L., Yang, C., Farhan, M., Chen, L., Su, Q., Zheng, W., 2022. Artemisinin attenuated ischemic stroke induced cell apoptosis through activation of ERK1/2/CREB/BCL-2 signaling pathway in vitro and in vivo. Int. J. Biol. Sci. 18, 457.

Salama, M., Ellaithy, A., Helmy, B., El-Gamal, M., Tantawy, D., Mohamed, M., et al., 2012. Colchicine protects dopaminergic neurons in a rat model of Parkinson's disease. CNS Neurol Disords 11, 836–843.

Salama, M., Sobh, M., Emam, M., Abdalla, A., Sabry, D., El-Gamal, M., et al., 2017. Effect of intranasal stem cell administration on the nigrostriatal system in a mouse model of Parkinson's disease. Exp. Ther. Med. 13, 976–982.

Shi, J.Q., Zhang, C.C., Sun, X.L., Cheng, X.X., Wang, J.B., Zhang, Y.D., Xu, J., Zou, H.Q., 2013. Antimalarial drug artemisinin extenuates amyloidogenesis and neuroinflammation in APPswe/PS1dE9 transgenic mice via inhibition of nuclear factor-κB and NLRP3 inflammasome activation. CNS Neurosci. Ther. 19, 262–268.

Teema, A.M., Zaitone, S.A., Moustafa, Y.M., 2016. Ibuprofen or piroxicam protects nigral neurons and delays the development of l-dopa induced dyskinesia in rats with experimental Parkinsonism: influence on angiogenesis. Neuropharmacology 107, 432–450.

Thirugnanam, T., Santhakumar, K., 2022. Chemically induced models of Parkinson's disease. Compar Biochem Physiol C Toxicol Pharmacol 252, 109213.

Tysnes, O.B., Storstein, A., 2017. Epidemiology of Parkinson's disease. J. Neural. Transm. 124, 901–905.

Wang, T., Li, C., Han, B., Wang, Z., Meng, X., Zhang, L., He, J., Fu, F., 2020. Neuroprotective effects of Danshensu on rotenone-induced Parkinson's disease models in vitro and in vivo. BMC Complement Med Therap 20, 1–10.

Wang, L., Wu, X., Yang, G., Hu, N., Zhao, Z., Zhao, L., Li, S., 2022. Cannabidiol alleviates the damage to dopaminergic neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease mice via regulating neuronal apoptosis and neuroinflammation. Neuroscience 498, 64–72.

Xiong, N., Yang, H., Liu, L., Xiong, J., Zhang, Z., Zhang, X., et al., 2013. bFGF promotes the differentiation and effectiveness of human bone marrow mesenchymal stem cells in a rotenone model for Parkinson's disease. Environ. Toxicol. Pharmacol. 36, 411–422.

Yan, J., Ma, H., Lai, X., Wu, J., Liu, A., Huang, J., et al., 2021. Artemisinin attenuated oxidative stress and apoptosis by inhibiting autophagy in MPP+-treated SH-SY5Y cells. J Biol Res (Greece) 28, 1–10.

Zeng, Z., Xu, J., Zheng, W., 2017. Artemisinin protects PC12 cells against β-amyloid-induced apoptosis through activation of the ERK1/2 signaling pathway. Redox Biol. 12, 625–633.

Zeng, X.S., Geng, W.S., Jia, J.J., 2018. Neurotoxin-induced animal models of Parkinson disease: pathogenic mechanism and assessment. ASN Neuro 10, 1759091418777438.

Zhao, X., Li, S., Gaur, U., Zheng, W., 2020. Artemisinin improved neuronal functions in Alzheimer' s disease animal model 3xtg mice and neuronal cells via stimulating the ERK/CREB signaling pathway. Aging Dis 11, 1–19.

Zheng, W., Chong, C.M., Wang, H., Zhou, X., Zhang, L., Wang, R., et al., 2016. Artemisinin conferred ERK mediated neuroprotection to PC12 cells and cortical neurons exposed to sodium nitroprusside-induced oxidative insult. Free Radical Biol. Med. 97, 158–167.