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Neuropharmacological Effects of Naringenin, Harmine and Adenosine on Parkinsonism Induced in Rats

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ABSTRACT

Parkinson's disease is characterized by dopaminergic-cell loss in the substantia nigra in the basal ganglia. Natural dietary antioxidants may exert protection against age-related deficits in cognitive and motor function. Present work conducted to evaluate the effects of some natural products in animal model of Parkinsonism which induced by rotenone injection (1.5 mg/kg s.c). Rats divided into 6 groups: - 1st control: rats injected with rotenone 6 doses every other day for 11 days, 2nd normal: rats injected i.p. with 1% cremophor, , injected s.c. DMSO. 3rd, 4th, 5th, 6th: rats given deprenyl (10 mg/kg, s.c), naringenin (50 mg/kg, p.o), harmine (5 mg/kg, i.p) & adenosine (500 mg/kg, i.p) 6 doses every other day for 11 days, 1 hour before rotenone injection. 24th after the last doses of all treatments, behavioural tests, rotarod and activity cage performed. At the end of the experiment, rats decapitated and brain removed for determination of brain neurotransmitters content as dopamine and its metabolite [dopamine (DA), 3,4 dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA)] and oxidative stress biomarkers as glutathione, malondialdehyde, nitric oxide (NOx) content. Results revealed that deprenyl and naringenin improved rats' locomotor activity, while, harmine and adenosine decreased the locomotor activity. Deprenyl, harmine, naringenin improved rats balancing time. Deprenyl and naringenin increased the dopamine content. Deprenyl, naringenin, harmine and adenosine treatment resulted in increased glutathione with decrease of malondialdehyde brain content. These findings suggested that all three tested agents improved the oxidative status induced by rotenone, however, naringenin and harmine counteracted the decrease in dopamine content.

Key words: Parkinsonism, rotenone, naringenin, harmine, adenosine, Rats

INTRODUCTION

Parkinson's disease (PD) is the most common neurodegenerative movement disorder with a prevalence of 0.5-1% in individuals of 65 -96 years and 1-3% among individuals of 80 years [55]. It is clinically characterized by four cardinal features: Resting tremors, postural instability, bradykinesia— these symptoms are attributed primarily but not exclusively to the selective loss of dopaminergic neurons in SNc (50-70% of dopaminergic neurons), which believed to be the most crucial [7, 13, 56]. Surviving neurons may contain Lewy bodies, intracytoplasmic protein aggregates mainly composed of α -synuclein and believed to be a second neuropathological feature of PD [64, 69]. The hypotheses for sporadic PD include combinations of the aging process, genetic propensity and environmental exposures hypothesis which posits that exposure to pesticides appears to correlate strongly with increased incidence of Parkinsonism [22, 23, 63], for example, The pesticide rotenone, a highly selective complex I inhibitor may produce neuropathological features of PD in rats [6, 67].

Based on several studies of cellular model analysis and in vitro experiment, it has become obvious that the toxic effect of rotenone is multifactorial; the insecticide might express its toxicity via the inhibition of complex I or the enhancement of activated microglia or the increased production of ROS or the increased oxidative damage of proteins, lipids and DNA [74]. Hence Rotenone is used in the current work as the induction model of PD. Rotenone is a commonly used organic pesticide extracted from *Leguminosae* plant and has a variety of known biological effects [54, 65].

Neuroprotection can be considered a form of therapy to slow the rate of progression of a neurodegenerative disease. Concepts of potential neuroprotective approaches for PD have developed over the last decade and focused on agents that reduce oxidative stress, combat excitotoxicity, enhance mitochondrial function, counteract inflammation and inhibit apoptosis. Currently available anti-parkinsonian agents exert several undesirable side effects. The development of safe and effective agents of natural origin may provide a better way to improve the patient's condition and lessen side effects.

Treatment with the plant extracts rich in polymethoxylated flavones, procyanidins and isoflavones (e.g. tangeretin peel and red clover) significantly attenuated the 6-OHDA-induced dopaminergic loss in rats [12]. There is evidence that some phenolics can cross BBB. Several animal studies claiming that monophenols (hesperitin and naringenin) can enter BBB [84]. However, polyphenols found in fruits such as blueberries are not only powerful antioxidants but can exert many other biological effects that may account for some of the neuroprotective actions. Altering stress signaling and neuronal communication, suggests that the dietary antioxidants may exert protection against pre-age related deficits in cognitive and motor function [35, 46].

Naringenin (4-oxo, 5,7-trihydroxy flavonone) is a plant bioflavonoid that belong to the class of flavonoids found in grapefruit [17]. Studies by Youdim *et al.* [80, 81] indicated that naringenin is able to traverse the BBB. Naringenin have already been pharmacologically evaluated as a potential antioxidant [61], through direct and indirect antioxidant activity corresponding to several steps in the cascade of oxidative events as well as neurodegenerative processes like Alzheimer's, dementia and Parkinson's diseases [33, 78].

Harmine is one of the β -carbolines which is a class of alkaloids, known as harmala alkaloids, bind with high affinity receptors in the brain such as 5-hydroxytryptamine receptor subtypes (5-HT₂, 5-HT_{1A}), imidazoline receptors and glutamate receptor of the type N-methyl-D-aspartate (NMDA) [14, 25, 47]. Therefore, B-Carbolines protect neurons against the excitotoxic effects of dopamine and glutamate [45], influence cerebral neurotransmitters [70] and display a protective effect on oxidative neuronal damage through a scavenging action on reactive oxygen species [38, 42, 58].

Adenosine is an endogenous purine nucleoside that modulates many physiological processes. Studies suggest that some degree of dopaminergic activity is needed to obtain adenosine antagonistic-induced motor activity. Furthermore, blockade of dopaminergic neurotransmission counteracts the antagonistic effect induced by adenosine [19]. Thus, it seems that monotherapy with A_{2A} antagonists may only be useful in the early stages of Parkinson's disease while selective antagonists of the adenosine A_{2A} receptors are widely used in treatment of Parkinson's disease [21, 36].

Therefore, it seemed interesting to test the neuroprotective effects of naringenin (50 mg/kg, p.o), harmine (5 mg/kg, i.p) and adenosine (500 mg/kg, i.p) in rotenone induced PD animal model. The effect of the three drugs was compared to that of deprenyl, a standard antiparkinsonian drug with known antioxidant properties. In order to achieve the goal of the present study, PD was induced in rats using rotenone (1.5 mg/kg, s.c) dissolved in DMSO injected on every other day for eleven days. Behavioral changes which might occur as a result of the induced disorder were recorded using the rotarod and the activity cage tests. Furthermore, dopamine content and its metabolites in rats' striata were estimated. Brain oxidative stress biomarkers namely, malondialdehyde, reduced glutathione and nitric oxide were also assessed.

MATERIALS AND METHODS

Animals:

Adult male albino Wister rats, weighing 180-200 g, obtained from the animal house of the National Research Center (Dokki, Giza, Egypt), were used in the current study. Rats were allowed at least one week of acclimatization before using them in the current experiments. All animals housed in plastic cages five per cage and kept in a conditioned

atmosphere at 25°C and 60% humidity. The animals were fed standard laboratory pellets (20% proteins, 5% fats and 1% multivitamins) with tap water ad libitum. Pellets were obtained from the animal house colony of the National Research Center (Dokki, Giza, Egypt). Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and Ethics Committee of Faculty of Pharmacy Cairo University.

Drugs and natural products:

All drugs, unless otherwise specified are purchased from Sigma Chemicals Co., St. Louis, MO, US. All test agents were freshly prepared in 1% camphor in normal saline either for subcutaneous, intraperitoneal or oral administration except for rotenone that was dissolved in dimethylsulfoxide (DMSO) for subcutaneous administration. The concentration was adjusted so that each 100 gm body weight received 1ml of drug suspension for oral administration and 0.2 ml for intraperitoneal and subcutaneous injections.

Experimental Design:**Behavioural training session:**

Rats were subjected to three days training sessions before injection of the tested drugs using rotarod in order to reach a stable performance on the rod, and on the fourth day rats were placed in the activity cage to habituate them to the apparatus and to record their basal activity [75].

Treatment:

On the fifth day of the training sessions, rats were divided into six groups, 15-20 rats each as following: 1st control (rotenone) group: rats were injected s.c. with rotenone (1.5mg/kg), 6 doses, every other day for eleven days to induce experimental PD [72]. 2nd normal group: rats were injected i.p. with 1% cremophor in normal saline, and injected s.c. with equivalent volume of DMSO. 3rd, 4th, 5th, 6th groups: rats were given deprenyl 10 mg/kg, s.c [40], naringenin 50 mg/kg, p.o [84], harmine 5 mg/kg, i.p [52] and adenosine 500 mg/kg, i.p [83] respectively 6 doses ,every other day for eleven days, 1 hour before rotenone injection.

Behavioral tests:

Behavioral tests namely rotarod for measurement of motor coordination and activity cage to test locomotor activity were performed to surviving rats twenty four hours after the last dose of the treatments.

- Rotarod test:

All rats used in the present study (except for outliers) were pre-trained on the rotarod apparatus in order to reach a stable performance (the average time in seconds spent on the rod). The training consisted of three sessions on three consecutive days before the injection of the tested drugs, where each session included three separate testing trials. Rats were first habituated to the stationary rod, and then accelerating speed was initiated at 4 rpm and increased gradually to reach 40 rpm over 300 seconds. By the last training session all selected rats had reached a stable rotarod performance and the average time spent on the rod in the three consecutive trials was used for the baseline calculation of rotarod performance [43]. Twenty four hours after the last dose of the tested drugs rats were then placed on the testing rod, starting at 4 rpm and accelerated linearly to 40 rpm over 300 seconds.

- Activity cage test:

The basal activity counts of rats were pretested in a 15 minutes interval the day before the experiment to habituate them to the apparatus; they were adapted for 5 minutes and the basal activity counts were then recorded for 10 minutes [37]. Twenty four hours after the last injection of the tested drugs, each rat was then exposed to the apparatus for 10 minutes test session. The arena was cleaned after each session [31]. Locomotor activity was calculated as the total rat activity counts during 10 minutes using grid floor detecting activity cage.

Brain tissue preparation: at the end of the experiment, twenty four hours after last injection of treatments rats were decapitated, their brains were carefully removed and both hemispheres were isolated according to the method described by [51]. Isolated striata were weighed and immediately frozen on dry ice then stored at -80°C. for determination of biochemical analysis and oxidative stress biomarkers

Biochemical analysis of the brain tissue homogenate: left striata homogenate was used for the determination of brain neurotransmitters [dopamine (DA), 3,4 dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA) contents] (Perkin-Elmer, USA). While, right striata homogenate was used for assessment of oxidative stress biomarkers [reduced glutathione, malondialdehyde and nitric oxide content] as follow:-

Estimation of brain neurotransmitters

Dopamine and its metabolite contents: dopamine and its metabolites (DOPAC and HVA) in striatal homogenate were determined according to the method described by Pagel *et al.*, [57]. Dopamine turnover was calculated according to the method described by Zbarsky *et al.*, [84].

Estimation of oxidative stress biomarkers:-**1. Glutathione content**

GSH in striatal homogenate was determined according to the method described by Ahmed *et al.*, [3].

2. Malondialdehyde content

Determination of lipid peroxides formation was measured in brain homogenate as thiobarbituric acid reactive substances (TBARS) according the method of Mihara and Uchiyama [49].

3. Nitric oxide (NOx) content

Nitric oxide was determined according to the method described by Miranda *et al.*, [50].

Statistical Analysis

All the values are presented as means \pm standard error of the means (mean \pm s.e). Comparison between more than two different groups was carried out using the parameteric one-way analysis of variance (ANOVA) followed by Tukey HSD multiple comparisons test. For behavioral parameters, square root transformed percent was calculated [34] then comparison between more than two different groups was carried out using the non-parameteric one-way analysis of variance (ANOVA) followed by Dunn's multiple comparisons test. Difference was considered significant when P is less than 0.05. SPSS software (version 7.5) and INSTAT software (version 2) were used to carry out these statistical tests.

RESULTS**1. Effect of naringenin, harmine and adenosine treatment in locomotor activity and motor coordination in rotenone-treated rats****Locomotor activity**

Subcutaneous injection of rotenone (1.5 mg/kg, s.c) for eleven days on every other day for a total of six injections significantly decreased the locomotor activity of rats reaching 34.57 % of their basal locomotor activity. Both deprenyl and naringenin significantly improved rats' locomotor activity to be 150 % and 130.6 % of that in the control (rotenone) group. While, both harmine and adenosine significantly decreased the locomotor activity of rats to be 72.5 % and 58.1 % of that in the rotenone control group (Table 1).

Motor coordination

Rotenone injection significantly decreased the balancing time of rats reaching 35.80 % of their basal balancing time. Simultaneous injection of deprenyl or harmine with rotenone increased rats' balancing time to 103.49 and 111.76 % of their basal balancing time respectively. On the other hand, naringenin (50 mg/kg, p.o) or adenosine injection together with rotenone decreased the basal balancing time of rats to be 64.31 and 56.7 % of their basal balancing time respectively. Both deprenyl and harmine significantly improved rats balancing time to be 171.18 and 177.96 % of that in the control group. While, both naringenin and adenosine didn't have a significant effect on the balancing time of rats as compared to control group (Table 2).

2. Effect of naringenin, harmine and adenosine on dopamine (DA), 3, 4 dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA) contents in the striata of rotenone-treated rats

Rotenone injection decreased dopamine content in the rats striata to 1.67 ± 0.09 $\mu\text{g/g}$ wet weight as compared to normal group, in which dopamine content was 7.29 ± 0.28 $\mu\text{g/g}$ wet weights. Similarly, DOPAC and HVA contents were reduced to 0.11 ± 0.007 and 0.024 ± 0.002 $\mu\text{g/g}$ wet weight respectively in the rotenone-treated rats, compared to that in the normal group which was 0.76 ± 0.03 and 0.046 ± 0.03 $\mu\text{g/g}$ wet weight respectively. On the other hand, dopamine turnover rate (TO), showed any significant changed in rotenone-treated rats when compared to the normal group .

Oral administration of naringenin , together with rotenone injection significantly increased dopamine content in rat striata to 518.56 % of the control group, while DOPAC and HVA contents didn't significantly change as compared to control group. On the other hand, dopamine turnover rate was significantly decreased to 13.75 % control group (Table 3). Also

administration of harmine simultaneously with rotenone injection was associated with a significant increase in dopamine content to 690.04 % and a significant decrease of dopamine turnover to 11.25 % of the control group. While dopamine metabolites didn't significantly change as compared to control group (Table 4). On the other hand, adenosine injected simultaneously with rotenone didn't induce any significant change in the level of dopamine, DOPAC, HVA nor the rate of dopamine turnover as compared to control group (Table 5).

3. Effect of naringenin, harmine and adenosine treatment on oxidative stress biomarkers in rotenone-treated rats

1. The effect on reduced glutathione content

Rotenone injection significantly decreased glutathione content to 0.026 ± 0.001 mg/g wet weight compared to normal group in which glutathione content was 0.075 ± 0.002 mg/g wet weight. Deprenyl injected simultaneously with rotenone, induced a significant increase in glutathione content to 223.07 % of the control group. Similarly, simultaneous administration of naringenin with rotenone injection showed a significant increase in glutathione content to 284.61% of the control group (Table 6). Administration either of harmine (Table 7) and adenosine (Table 8) simultaneously with rotenone significantly increased glutathione content to 211.53 and 326.92 % respectively of control group.

2. The effect on malondialdehyde (MDA) content:

Injection of rotenone (1.5 mg/kg, s.c) for eleven days on every other day for a total of six injections significantly increased malondialdehyde content to 146.35 ± 11.38 nmol/ml wet weight compared to normal group in which malondialdehyde content was 40.75 ± 4.33 nmol/ml wet weight. The injection of deprenyl (10 mg/kg, s.c) simultaneously with rotenone induced a significant decrease in malondialdehyde content in the rat striata to 31.02 % of control group. Similarly, simultaneous administration of naringenin (50 mg/kg, p.o), harmine (5mg/kg, i.p) and adenosine (500mg/kg, i.p) with rotenone showed a significant decrease in malondialdehyde content to 54.92, 29.44 and 82.36 % respectively compared to that in the control group (Table 6,7,8).

3. The effect on nitric oxide (NOx) content:

Rotenone subcutaneously injected at a dose of (1.5 mg/kg) for eleven days on every other day for a total of six injections didn't significantly decrease nitric oxide content compared to normal group. Deprenyl (10 mg/kg, s.c) simultaneously injected with rotenone didn't affect nitric oxide content in the rat striata as compared to control group. In addition, combination of rotenone with any of the three agents didn't induce any significant change in nitric oxide level as compared to control group. On the other hand, deprenyl, naringenin, harmine and adenosine as compared to normal group (Table 6, 7, 8).

DISCUSSION

In the present study, injection of rotenone (1.5 mg/kg, s.c) into rats for eleven days on every other day for a total of six injections resulted in a significant decrease in locomotor activity tested by the activity cage. These findings are in agreement with Fleming *et al.* [20]. Also, rotenone treated rats, induced a decrease in the time spent on the rotarod and these results are in accordance with a previous study that showed that rotenone (1.5 mg/kg, s.c) injected every other day for eleven days showed a decrease in muscle coordination and stayed for a shorter period on rotarod as compared to the normal animals [16]. As regard striatal dopamine concentrations rotenone injection induced a decrease to 22.9 % and these findings are in consistent with those obtained by Abd El-Gawad *et al.* [1]. Concerning dopamine metabolites (DOPAC and HVA), results of the present study showed a significant decrease in DOPAC and HVA levels in striatum after rotenone treatment while ,DA turnover (the ratio DOPAC+ HVA/DA) wasn't significantly changed. These results may be attributed to a defect in substantia nigra pars compacta (SNc) that may result in a decreased capacity to release DA in the striatum and thus a decrease in its metabolites content [79]. In the present study, striatal GSH content was reduced to 34.66 % in the rotenone treated group as compared to the normal group. This finding is in harmony with a previous study reporting that rotenone induced about 49 % decrease in the GSH levels in substantia nigra and 26% decrease in nucleus caudatus putamen on the fifth day of rotenone injection [62]. Rotenone treated rats in current study showed a marked increase in the tissue MDA content, the decomposition product of lipid peroxidation and these results in agreement with Bashkatova *et al.* [5]. As regard NO content assessment in the striatal tissue showed no change and these findings are in harmony with Cutillas *et al.* [11] and Gao *et al.* [23]. Fleming and his co-workers,[20] suggested that it is possible that rotenone may affect dopamine transporter activity and influence the expression and activity of striatal enzymes involved in dopamine synthesis. Therefore, it is possible that the motor abnormalities observed following rotenone treatment in the current study

were caused by alterations in the nigrostriatal dopaminergic system; they also speculated that other systems may be affected. The understanding of the mechanisms underlying rotenone-induced dopaminergic neuronal death could provide some insights into the processes responsible for the selective neurodegeneration of nigrostriatal dopaminergic neurons in PD patients. It is widely believed that increased oxidative stress and mitochondrial dysfunction contribute to dopaminergic neuronal degeneration. Although dopaminergic neurons are more sensitive to rotenone toxicity than other neurons [2, 60]. These observations are consistent with other report demonstrating that energy deprivation rather than ROS may be a main mechanism of rotenone-induced cell death in dopaminergic neurons [41]. The results of the present study revealed that deprenyl (10 mg/kg, s.c) injected in rotenone-treated rats reversed bradykinesia and prolonged the time spent on the rod. Similarly, Engberg *et al.* [18] who reported that administration of deprenyl (3-30 mg/kg, i.p.) to normal rats caused a dose-dependent increase in the spontaneous locomotor activity. The improvement in rats' behavior following deprenyl treatment could be explained through its capacity to protect dopaminergic neurons from the toxic effects of rotenone and the increase in dopamine content [29]. Deprenyl was initially used in the treatment of PD based on its ability to exert its neuroprotective effect through reducing the catabolism of dopamine by inhibition of MAO-B enzyme. Thereby, increasing dopamine level with a subsequent increased activity on D2 receptor [76]. In the current results, deprenyl induced a significant increase in dopamine content in striatum of rotenone treated rats accompanied with non significant change in dopamine metabolites (DOPAC and HVA). This finding is in accordance with Knoll [39]. The increase in dopamine content obtained with deprenyl can be discussed on the basis of inhibition of the uptake of DA [85] which may be due to the high concentration of the *R*-(2)-amphetamine and desmethylelegriline metabolites of deprenyl or the accumulation of an endogenous factor such as beta-phenylethylamine, both are potent uptake inhibitors and have been suggested to play a role in the protective effects of deprenyl against MPTP and 6-OHDA toxicity *in vivo* [4, 27,71]. The results of the present study also revealed that administration of deprenyl (10 mg/kg, s.c) in rotenone-treated rats reversed the oxidative stress induced by rotenone observed through an increase of glutathione and reduced MDA content in rats striata. This result is in accordance with Olanow *et al.* [56]. The neuroprotective effect of deprenyl might be due to its ability to prevent free radical formation, enhance the activity of antioxidant enzymes such as SOD and CAT, protects against peroxynitrite and nitric oxide induced apoptosis by up-regulation of antiapoptotic and antioxidant molecules, in addition of being a free radical scavenger [15, 53, 66, 77]. In the current work, naringenin (50 mg/kg, p.o) significantly increased locomotor activity of rats but didn't affect motor coordination of rotenone-treated rats as compared to control groups. In addition, it induced significant increase in dopamine content in striatum to 518.56 % while dopamine metabolites (DOPAC and HVA) didn't significantly change as compared to control group. Naringenin significantly increased glutathione level, decreased MDA level, while no change in nitric oxide contents compared to control group. It was reported that certain flavanones, hesperitin and naringenin were able to pass the BBB and exert a useful chemopreventive action against neurodegenerative disease [30, 81]. Beyond the very potent ROS direct scavenger action, flavonoids exert an important indirect antioxidant activity contributing to the homeostasis of calcium, metal chelation, stabilisation of membranes through anti-lipoperoxidation and enzymatic activity modulation [8,10, 24]. In addition, they have anti-inflammatory properties which can modulate both neurodegenerative and vascular diseases [48, 59]. In the present study, injection of harmine (5mg/kg, i.p) in rats didn't reverse hypomotility induced by rotenone while, motor coordination was significantly improved as compared to control group. In agreement with these results is [68]. In the present study, injection of harmine (5 mg/kg, s.c) induced an increase in dopamine to 158.16 % while there was no significant change in dopamine metabolites level, these results are in accordance with those previously reported by Iurlo *et al* [32] While it increased the reduced glutathione to 211.53 % and decreased MDA content to 29.44 % as compared to control (rotenone) group, without effects on nitric oxide level. These findings are in agreement with Young Su Han *et al* [82] who stated that harmine increased GSH content *in vitro*. Several studies revealed that beta-carbolines have effective antioxidant abilities by its radical scavenging properties. They are found to inhibit lipid peroxidation of liver microsomes [73] and to attenuate oxidative damage of hyaluronic acid, cartilage collagen and immunoglobulin G [38]. In addition, it was shown that harmalol had a protective effect against MPTP-induced neurotoxicity in the mouse [42]. Adenosine treatment in current study didn't improve the behavior of rotenone-treated rats and these effects can be explained by that adenosine plays a role opposite to dopamine in the striatum. Both dopamine antagonists and adenosine agonists produce similar effects in different behavioural tests. This was first pointed out by Heffner *et al.* [28], who found that some adenosine analogues attenuated spontaneous locomotor activity and didn't impair motor coordination in mice. Current study revealed that injection of adenosine (500 mg/kg, i.p) in rats treated with rotenone showed no change in dopamine and its metabolites levels in striatum compared to rotenone control. While it induced increase in glutathione levels and decrease in MDA levels while didn't alter nitric oxide content in rats treated with rotenone. These results in harmony with Zafar *et al.* [83] who reported that treating rats with different doses of adenosine decreased the levels of lipid peroxides and elevated the level of reduced glutathione

in substantia nigra of 6-OHDA lesioned rats. It has been reported that adenosine acts as an endogenous activator of the cellular antioxidant system [26, 44] and inhibits the superoxide anion radical generation by neutrophils [9,26] and has scavenger properties against hydroxyl free radical.

Table (1): Effect of naringenin, harmine and adenosine on locomotor activity tested by using activity cage

Parameters Treatment	Locomotor activity			
	Activity Count		% of basal activity	
	Before treatment	After treatment	Percentage	Square-root- transformed %
Normal group (vehicle)	175.12±20.97	174.62±5.85	99.7%	0.98 ± 0.04
Control (rotenone) (1.5 mg/kg, s.c)	180.37±24.82	62.37±2.21	34.57%	0.62 ± 0.04*
Deprenyl (10mg/kg, s.c) +rotenone (1.5mg/kg, s.c)	234.62±15.39	199.12±14.10	84.86%	0.93 ± 0.05 [®]
Naringenin (50mg/kg, p.o) +rotenone (1.5mg/kg, s.c)	229.13±12.45	147.00±11.28	64.19%	0.81 ± 0.05 [®]
Harmine (5mg/kg, i.p) +rotenone (1.5 mg/kg, s.c)	148.62±5.54	29.87±7.21	20.1%	0.45 ± 0.01 ^{®*}
Adenosine (500mg/kg, i.p) +rotenone (1.5 mg/kg, s.c)	297.50±24.67	37.12±6.15	12.47%	0.36 ± 0.02 ^{®*}

* Significantly different from the corresponding normal group values at $p < 0.05$.

[®] Significantly different from the corresponding control (rotenone) group values at $p < 0.05$

Rats were given rotenone (1.5 mg/kg, s.c) either alone or simultaneously with deprenyl (10 mg/kg, s.c), naringenin (50 mg/kg, p.o), harmine (5 mg/kg, i.p) or adenosine (500 mg/kg, i.p) for eleven days on every other day for a total of six injections. Data was expressed as mean of 15 animals ± SE. Statistical analysis was carried out by one way ANOVA followed by Dunn's multiple comparisons test.

Table (2): Effect of naringenin, harmine and adenosine on motor coordination tested by using rotarod in rotenone-treated rats

Parameters Treatment	Balancing time on the Rotarod			
	Balancing time		% of basal time	
	Before treatment	After treatment	Percentage	Square-root- transformed %
Normal group (vehicle)	234.37±17.53	235.54±16.30	100.5 %	1.01 ± 0.01
Control (rotenone) (1.5 mg/kg, s.c)	217.50±11.40	77.87±13.32	35.80 %	0.59 ± 0.03*
Deprenyl (10mg/kg, s.c) +rotenone (1.5mg/kg, s.c)	202.83±14.17	209.91±21.30	103.49 %	1.01 ± 0.05 [®]
Naringenin (50mg/kg, p.o) + rotenone (1.5mg/kg, s.c)	167.45±15.16	107.70±5.97	64.31 %	0.82 ± 0.06
Harmine (5mg/kg, i.p) +rotenone (1.5 mg/kg)	152.66±5.77	170.62±17.80	111.76 %	1.05 ± 0.06 [®]
Adenosine (500mg/kg, i.p) +rotenone (1.5 mg/kg, s.c)	221.25±19.85	125.45±35.77	56.7 %	0.75 ± 0.09

* Significantly different from the corresponding normal group values at $p < 0.05$.

[®] Significantly different from the corresponding control (rotenone) group values at $p < 0.05$.

Rats were given rotenone (1.5 mg/kg, s.c) either alone, with deprenyl (10 mg/kg, s.c), naringenin (50 mg/kg, p.o), harmine (5 mg/kg, i.p) or adenosine (500mg/kg,i.p) for eleven days on every other day for a total of six injections. Data was expressed as mean of 15 animals±SE. Statistical analysis was carried out by one way ANOVA followed by Dunn's multiple comparisons test.

Table (3): Effect of naringenin on dopamine (DA), 3,4 dihydroxy-phenylacetic acid (DOPAC), homovanilic acid (HVA) contents and its turnover rate (TO) in the striata of rotenone-treated rats

Parameters Treatment	DA(ug/gm)	DOPAC(ug/gm)	HVA(ug/gm)	TO
Normal group (vehicle)	7.29±0.28	0.76±0.03	0.046±0.03	0.11±0.008
Control(rotenone) (1.5 mg/kg,s.c)	1.67±0.09*	0.11±0.007*	0.024±0.002*	0.08±0.006
Deprenyl (10 mg/kg,s.c) +rotenone (1.5 mg/kg,s.c)	4.27±0.11@*	0.31±0.034*	0.027±0.002*	0.07±0.008
Naringenin (50 mg/kg,p.o) +rotenone (1.5mg/kg,s.c)	8.66±0.52@	0.07±0.006*	0.027±0.002*	0.011±0.0003@*

*Significantly different from the corresponding normal group values at $p<0.05$.

@ Significantly different from the corresponding control (rotenone) group values at $p<0.05$.

TO=turnover= rate of dopamine turnover calculated as $(HVA+DOPAC)/DA$

Rats were given rotenone (1.5 mg/kg, s.c) either alone or simultaneously with deprenyl (10 mg/kg, s.c) or naringenin (50 mg/kg, p.o) for eleven days on every other day for a total of six injections. Data was expressed as mean of 6 animals ± SE. Statistical analysis was carried out by one way ANOVA followed by Tukey HSD multiple comparisons test.

Table (4): Effect of harmine on dopamine (DA), 3,4 dihydroxy-phenylacetic acid (DOPAC), homovanilic acid (HVA) contents and its turnover rate (TO) in the striata of rotenone-treated rats

Parameters Treatment	DA(ug/gm)	DOPAC(ug/gm)	HVA(ug/gm)	TO
Normal group (vehicle)	7.29±0.28	0.76±0.03	0.046±0.03	0.11±0.008
Control(rotenone) (1.5 mg/kg,s.c)	1.67±0.09*	0.11±0.007*	0.024±0.002*	0.08±0.006
Deprenyl (10 mg/kg,s.c) +rotenone (1.5 mg/kg,s.c)	4.27±0.11@*	0.31±0.034*	0.027±0.002*	0.07±0.008
Harmine (5mg/kg,i.p) +rotenone (1.5 mg/kg)	11.53±1.00@*	0.10±0.01*	0.015±0.002*	0.009±0.001@*

*Significantly different from the corresponding normal group values at $p<0.05$.

@ Significantly different from the corresponding control (rotenone) group values at $p<0.05$.

TO=turnover= rate of dopamine turnover calculated as $(HVA+DOPAC)/DA$.

Rats were given rotenone (1.5 mg/kg, s.c) either alone or simultaneously with deprenyl (10 mg/kg, s.c) or harmine (5 mg/kg, i.p) for eleven days on every other day for a total of six injections. Data was expressed as mean of 6 animals±SE. Statistical analysis was carried out by one way ANOVA followed by Tukey HSD multiple comparisons test.

Table (5): Effect of adenosine on dopamine (DA), 3,4 dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA) contents and its turnover rate (TO) in the striata of rotenone-treated rats

Parameters Treatment	DA (ug/gm)	DOPAC (ug/gm)	HVA (ug/gm)	TO
Normal group (vehicle)	7.29±0.28	0.76±0.03	0.046±0.03	0.11±0.008
Control(rotenone) (1.5 mg/kg,s.c)	1.67±0.09*	0.11±0.007*	0.024±0.002*	0.08±0.006
Deprenyl (10 mg/kg,s.c) +rotenone (1.5 mg/kg,s.c)	4.27±0.11 [®] *	0.31±0.034*	0.027±0.002*	0.07±0.008
Adenosine (500mg/kg,i.p) +rotenone (1.5 mg/kg,s.c)	1.96±0.13*	0.13±0.005*	0.038±0.002	0.085±0.007

*Significantly different from the corresponding normal group values at $p < 0.05$.[®] Significantly different from the corresponding control (rotenone) group values at $p < 0.05$.TO=turnover= rate of dopamine turnover calculated as $(HVA+DOPAC)/DA$

Rats were given rotenone (1.5 mg/kg, s.c) either alone or simultaneously with deprenyl (10 mg/kg, s.c) or with adenosine (500 mg/kg, i.p) for eleven days on every other day for a total of six injections. Data was expressed as mean of 6 animals \pm SE. Statistical analysis was carried out by one way ANOVA followed by Tukey HSD multiple comparisons test.

Table (6): Effect of naringenin on glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NOx) content in striata of rotenone-treated rats

Parameters Treatment	GSH (mg/ml)	MDA (nmol/ml)	NO (μ mol/l)
Normal group (vehicle)	0.075±0.002	40.75±4.33	40.17±4.02
Control(rotenone) (1.5 mg/kg,s.c)	0.026±0.001*	146.35±11.38*	29.44±3.60
Deprenyl (10 mg/kg,s.c) +rotenone (1.5 mg/kg,s.c)	0.058±0.007 [®]	45.40±3.01 [®]	16.92±0.57*
Naringenin (50mg/kg,p.o) +rotenone (1.5 mg/kg,s.c)	0.074±0.01 [®]	80.38±2.85 ^{®*}	19.57±2.54*

*Significantly different from the corresponding normal group values at $p < 0.05$.[®] Significantly different from the corresponding control (rotenone) group values at $p < 0.05$

Rats were given rotenone (1.5 mg/kg, s.c) either alone or simultaneously with deprenyl (10 mg/kg, s.c) or with naringenin (50 mg/kg, p.o) for eleven days on every other day for a total of six injections. Data was expressed as mean of 6-8 animals \pm SE. Statistical analysis was carried out by one way ANOVA followed by Tukey HSD multiple comparisons test.

Table (7): Effect of harmine on glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NOx) content in striata of rotenone-treated rats

Parameters Treatment	GSH (mg/ml)	MDA (nmol/ml)	NO ($\mu\text{mol/l}$)
Normal group (vehicle)	0.075 \pm 0.002	40.75 \pm 4.33	40.17 \pm 4.02
Control(rotenone) (1.5 mg/kg.s.c)	0.026 \pm 0.001*	146.35 \pm 11.38*	29.44 \pm 3.60
Deprenyl (10 mg/kg.s.c) +rotenone (1.5 mg/kg.s.c)	0.058 \pm 0.007 [®]	45.40 \pm 3.01 [®]	16.92 \pm 0.57*
Harmine (5mg/kg.i.p) +rotenone (1.5 mg/kg.s.c)	0.055 \pm 0.004 [®]	43.10 \pm 1.15 [®]	19.14 \pm 0.96*

*Significantly different from the corresponding normal group values at $p < 0.05$.[®] Significantly different from the corresponding control (rotenone) group values at $p < 0.05$.

Rats were given rotenone (1.5 mg/kg, s.c) either alone or simultaneously with deprenyl (10 mg/kg, s.c) or with harmine (5 mg/kg, i.p) for eleven days on every other day for a total of six injections. Data was expressed as mean of 6-8 animals \pm SE. Statistical analysis was carried out by one way ANOVA followed by Tukey HSD multiple comparisons test.

Table (8): Effect of adenosine on glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NOx) content in striata of rotenone-treated rats

Parameters Treatment	GSH (mg/ml)	MDA (nmol/ml)	NO ($\mu\text{mol/l}$)
Normal group (vehicle)	0.075 \pm 0.002	40.75 \pm 4.33	40.17 \pm 4.02
Control(rotenone) (1.5 mg/kg.s.c)	0.026 \pm 0.001*	146.35 \pm 11.38*	29.44 \pm 3.60
Deprenyl (10 mg/kg.s.c) +rotenone (1.5 mg/kg.s.c)	0.058 \pm 0.007 [®]	45.40 \pm 3.01 [®]	16.92 \pm 0.57*
Adenosine (500mg/kg.i.p) +rotenone (1.5 mg/kg.s.c)	0.085 \pm 0.01 [®]	120.54 \pm 5.85 ^{*®}	19.35 \pm 1.49*

*Significantly different from the corresponding normal group values at $p < 0.05$.[®] Significantly different from the corresponding control (rotenone) group values at $p < 0.05$.

Rats were given rotenone (1.5 mg/kg, s.c) either alone or simultaneously with deprenyl (10 mg/kg, s.c) or with adenosine (500 mg/kg, i.p) for eleven days on every other day for a total of six injections. Data was expressed as mean of 6-8 animals \pm SE. Statistical analysis was carried out by one way ANOVA followed by Tukey HSD multiple comparisons test.

CONCLUSION

In this study, the ability of naringenin and harmine to ameliorate rotenone-induced behavioral and biochemical disturbance add weight to the evidence supporting the pathologic role of dopamine and oxidative stress in the development of parkinsonism.

So, the present findings suggested that all three tested agents improved the oxidative status induced by rotenone. However, naringenin and harmine counteracted the decrease in dopamine content an effect that was reflected on the rats' behavior.

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