

## Protective Role of Apigenin Against Rotenone Induced Model of Parkinson's Disease: Behavioral Study

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### ABSTRACT

Rotenone is a pesticide that has been shown to cause degeneration of dopaminergic neurons within the substantianigra and striatum. In this study, we investigated the protective effects of Apigenin on rotenone induced dopaminergic neurotoxicity. Adult male wistar albino rats were injected with rotenone (6µg/1µl in DMSO) or vehicle (1µl DMSO) into the right substantianigra pars compacta (SNpc) under stereotaxic surgery. Two varying doses of Apigenin (10 mg/kg and 20 mg/kg/day, i.p.) were administered for 14 days. The significant increase in catalepsy, postural instability, impaired motor coordination, decrease in locomotor activity, and the decrease in rearing behavior caused due to rotenone induction was attenuated by treatment with varying doses of apigenin. These results suggest that apigenin may serve as a promising therapeutic tool in Parkinson's disease.

**Keywords:** Rotenone, Apigenin, Substantia nigra, Catalepsy, Postural instability.

### INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. It is characterized by tremor at rest, rigidity, bradykinesia, hypokinesia and postural instability. Pathological features of PD include loss of nigrostriatal dopamine neurons in the Substantianigra pars compacta (SNpc) and the presence of Lewy bodies in the surviving dopamine neurons<sup>1-3</sup>. The pathogenesis of Parkinson's disease (PD) center on the formation of reactive oxygen species and the onset of oxidative stress leading to oxidative damage to substantianigra pars compacta<sup>4</sup>. A profusion of reactive microglia is seen in the substantianigra (SN) and striatum, not only in idiopathic PD but also in familial PD. Production of inflammatory products by these microglial cells characterizes the slow destructive process in P<sup>5-6</sup>. Rotenone (ROT) is a classical mitochondrial complex-I inhibitor and is the most potent member of the rotenoids, a family of isoflavonoids extracted from Leguminosae plants<sup>7</sup>. It leads to excessive production of ROS giving rise to severe oxidative stress. Rotenone is extremely hydrophobic, and it crosses biological membranes easily for access to the cytoplasm of dopaminergic neurons<sup>9,10</sup>. Systemic administration of rotenone has been used as a relevant rodent model for Parkinson's disease<sup>8</sup>. Several researchers intracerebrally infused rotenone into the median forebrain bundle (MFB)<sup>11,12</sup> or into the substantianigra pars compacta (SNc)<sup>13</sup>, but these models failed to reproduce the pathological features of hemiparkinsonism in rats. Apigenin (4', 5, 7-trihydroxyflavone) is a member of the flavone subclass of flavonoids present in fruits and

vegetables such as onions, oranges, parsley, and chamomile<sup>14</sup>. Apigenin exhibits a variety of biological effects, including anti-carcinogenic, anti-inflammatory, and free radical-scavenging activities<sup>15</sup>. Recent studies suggest that flavonoids prevent and delay neurodegeneration, in aged population cognitive dysfunction, mood decline and oxidative pathologies<sup>16</sup>. Antioxidants are now being used as drug candidates for treating pathologies related to free radical oxidation<sup>17</sup>. Our goal in the present study was to determine whether rotenone exerts any behavioral deficits in unilaterally rotenone lesioned rats. Behavioral parameters like catalepsy, postural instability, akinesia and motor coordination were used.

### MATERIALS AND METHODS

Chemicals Rotenone and Apigenin were purchased from Sigma (India). Animals and Treatments Healthy adult male albino rats of Wistar strain *Rattus norvegicus* weighing 250–300 g were housed in clean polypropylene cages and maintained in an air conditioned animal facility with constant 12 h/12 h dark and light cycle. All animal procedures were approved by our Institute of Ethical Committee. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) (IAEC No. 01/11/2013) Dr. ALMPGIBMS, University of Madras, Taramani campus, Chennai 113, Tamil Nadu, India. Rats were divided into five experimental groups of six animals each. Group I: sham-operated control received 2µl of vehicle (PEG/DMSO) through intranigral injection. Group II: Rats were given rotenone (6µg/ 1µl of PEG/DMSO) through intranigral injection on 1<sup>st</sup> day.

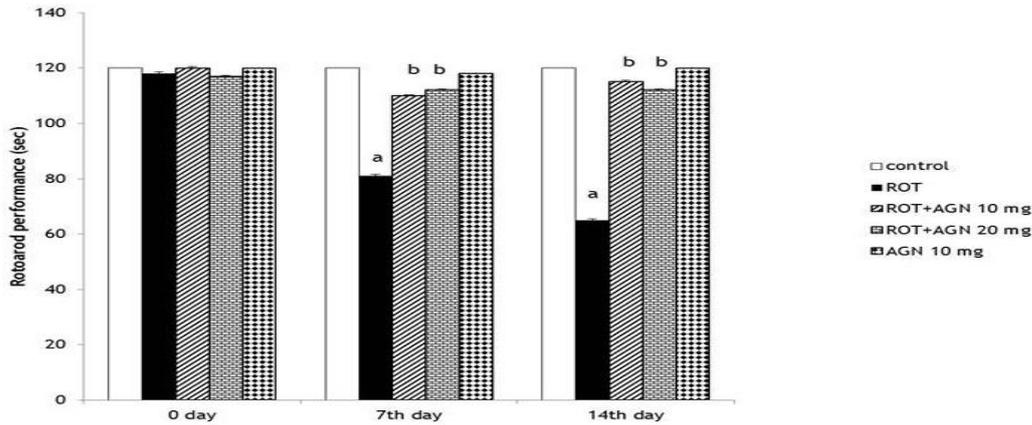


Figure 1: Effect of Apigenin on rotarod activity in Rotenone lesioned rats. Data presented are mean  $\pm$  SD (n=6). <sup>a</sup> $P < 0.01$  versus vehicle-treated group, <sup>b</sup> $P < 0.01$  versus rotenone treated group, (one-way ANOVA followed by Tukey's post hoc test).

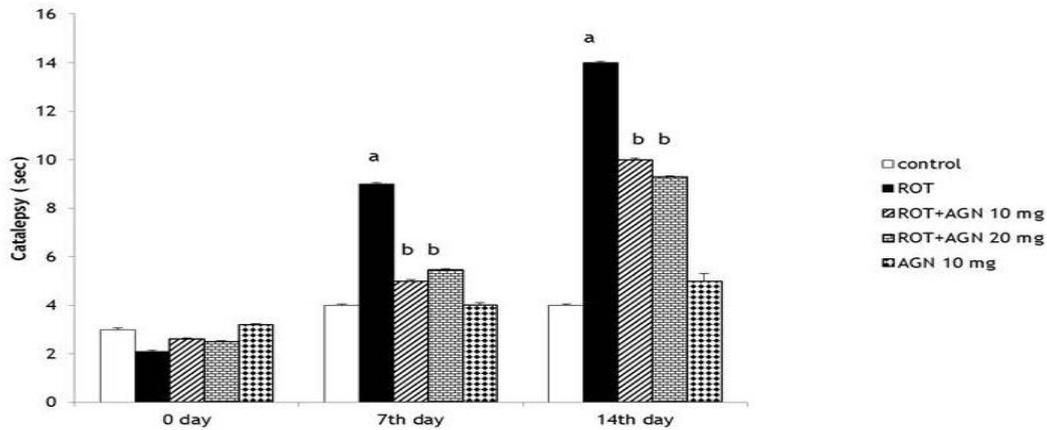


Figure 2: Effect of Apigenin on Rotenone induced cataleptic behavior. Data presented are mean  $\pm$  SD (n=6). <sup>a</sup> $P < 0.01$  versus vehicle-treated group, <sup>b</sup> $P < 0.05$  versus rotenone treated group, (one-way ANOVA followed by Tukey's post hoc test).

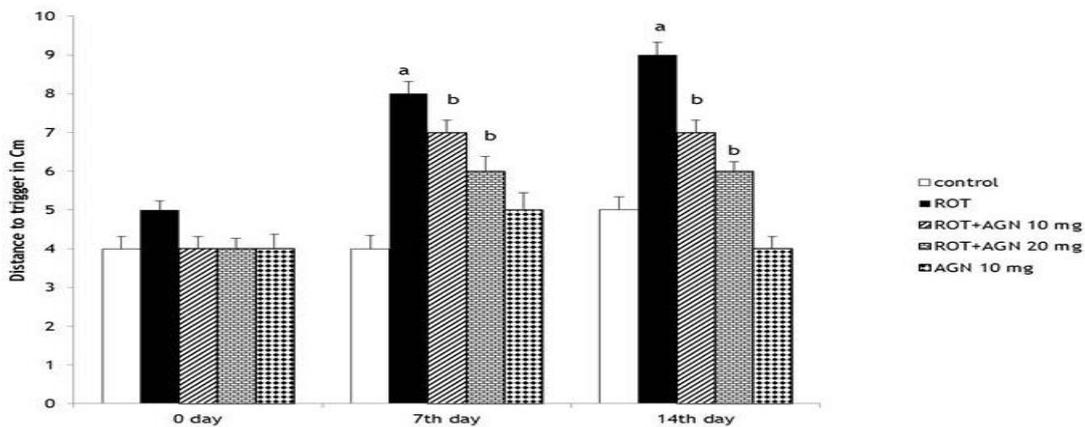


Figure 3: Effect of Apigenin on Rotenone induced postural instability. Data presented are mean  $\pm$  SD (n=6). <sup>a</sup> $P < 0.01$  versus vehicle-treated group, <sup>b</sup> $P < 0.05$  versus rotenone treated group, (one-way ANOVA followed by Tukey's post hoc test).

Group III: Rats were given rotenone (6 $\mu$ g/ 1 $\mu$ l of PEG/DMSO) through intranigral injection on 1<sup>st</sup> day followed by intraperitoneal administration of apigenin

(10mg /kg in PEG/DMSO) for 14days. Group IV: Rats were given rotenone (6 $\mu$ g/ 1 $\mu$ l of PEG/DMSO) through intranigral injection on 1<sup>st</sup> day followed by intraperitoneal

administration of apigenin (20 mg /kg in PEG/DMSO) for 14 days. Group V: Rats were given apigenin (20mg/kg in PEG/DMSO) intraperitoneally for 14 days. Intranigral Rotenone administration Rats were anaesthetized with ketamine and xylazine intraperitoneally and placed on a small animal stereotaxic frame (Instruments and Chemicals, Ambala, New Delhi). Rotenone dissolved in DMSO:PEG (1:1) was injected into the right SNpc at a flow rate of 0.2  $\mu$ l/min. The stereotaxic coordinates were: lateral = 0.20; antero-posterior = 0.53; and dorso-ventral = 0.75, from the Bregma point [18]. In sham control animals, 2  $\mu$ l of the vehicle (DMSO and PEG in the ratio of 1:1) was infused into the right SNpc. Proper post-operative care was taken till the animals recovered completely. Behavioral studies Behavioral assessments were carried out before the start of the treatment, then regularly at an interval of 7 days post treatment and final behavioral quantification was done after 24 h of last dose. Rotarod activity Motor incoordination and grip strength was assessed by using rotarod apparatus (Instruments and Chemicals, Ambala, New Delhi). Animals were exposed to prior training session to acclimatize them on rotarod

before starting the actual assessing of drug treatment. Animals were placed on the rotating rod with a diameter of 7 cm (speed 10 rpm). The cut off time was 180 s. Three separate trials after 5min gap were given to each rat. The average fall of time was recorded and expressed as count per 5min. Catalepsy Catalepsy was evaluated using the bar test in which, the rats were placed in half rearing position with both the front paws on a horizontal bar, 9 cm above and parallel to the base. Rats were observed with a stopwatch to note the time of removal of one paw from the bar. The maximum cut off time for observation was fixed at 180s<sup>19</sup>. Rearing behavior When placed in a clear cylinder, rats will engage in exploratory behavior, including rearing. This behavior was observed in the present test by placing the rat in a clear plexi glass cylinder (height 30 cm, diameter 20 cm) for 5 min, and quantifying the number of rears. Rear was classified, when the animal raised his forelimbs above shoulder level, and make contact with the cylinder wall with either one or both forelimbs. Removal of both forelimbs from the cylinder wall and contact with the table surface was required before another rear was scored<sup>20</sup>. Postural instability test To

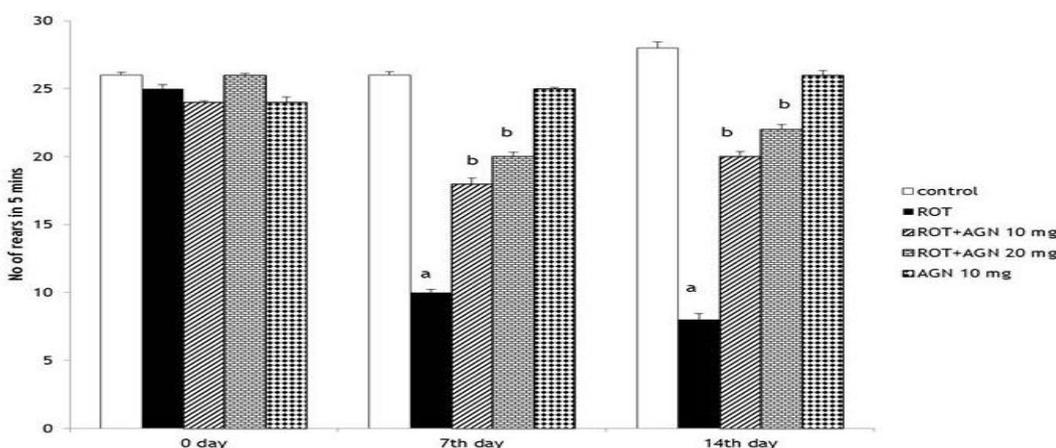


Figure 4: Effect of Apigenin on Rotenone induced rearing behavior. Data presented are mean  $\pm$  SD (n=6). <sup>a</sup>*P* < 0.01 versus vehicle-treated group, <sup>b</sup>*P* < 0.05 versus rotenone treated group, (one-way ANOVA followed by Tukey's post hoc test).

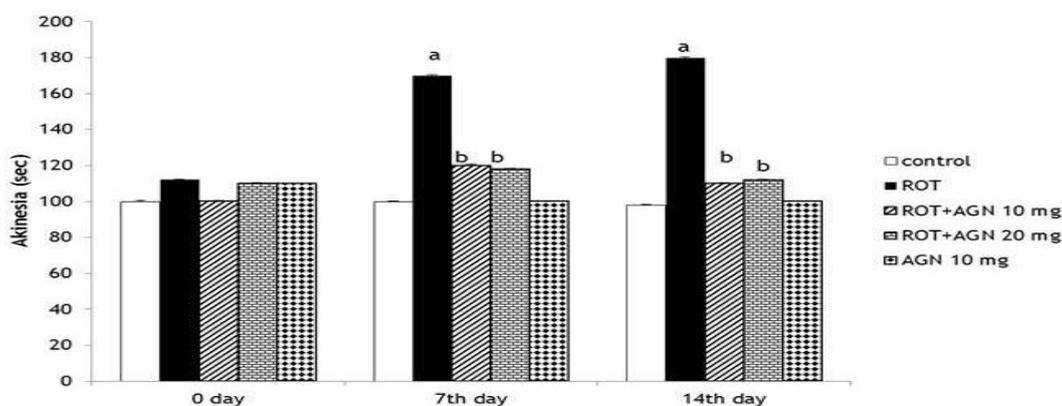


Figure 5: Effect of Apigenin on Rotenone induced akinesia. Data presented are mean  $\pm$  SD (n=6). <sup>a</sup>*P* < 0.01 versus vehicle-treated group, <sup>b</sup>*P* < 0.05 versus rotenone treated group, (one-way ANOVA followed by Tukey's post hoc test).

quantify postural instability, which is a hallmark feature of PD, modified method of Cannon et al<sup>20</sup> is used. In this test, rat was held vertically, while one forelimb was allowed to contact the table surface, which was lined with medium-grit sand paper. The rat's center of gravity was then advanced until the rat initiated a "catch-up" step. The displacement distance required for the rat to regain the center of gravity was recorded. At each time-point, three trials for each forelimb were recorded and the average reported. Akinesia This was measured as described previously by noting the latency in seconds (s) of the animals to move all four limbs and the test was terminated if the latency exceeded 180s<sup>21</sup>. Each animal was initially acclimatized for 5 min on a wooden elevated (30 cm) platform (40 cm × 40 cm). Using a stopwatch, the time taken (s) by the animal to move all the four limbs was recorded. This exercise was repeated five times for each animal. Statistical analysis Data of all the results were presented as mean ± SEM. The analysis of all the studies were done with the help of analysis of variance (ANOVA) followed by Tukey's post hoc test. A value of  $p < 0.05$  was considered to be statistically significant.

## RESULTS

Effect of apigenin in the behavioral deficits induced unilateral intranigral rotenone lesion Rotarod Rotenone induction significantly impaired muscle coordination ( $p < 0.01$ ) as compared to sham control group. Apigenin treatment (10mg/kg and 20mg/kg) significantly improved muscle grip strength (delayed fall of time) as compared to rotenone treated group ( $p < 0.01$ ) (Fig.1). However, 20 mg/kg dose of apigenin did not produce any significant effect on grip strength performance as compared to vehicle treated group. Catalepsy Time of removal of one front paw from the bar was noted and then averaged. On 14<sup>th</sup> day of treatment there was significant increase in catalepsy in rotenone treated groups compared to vehicle treated group ( $p < 0.01$ ). This change in cataleptic behavior was significantly reversed in apigenin (10 mg/kg and 20 mg/kg) treated group ( $p < 0.05$ ) (Fig.2). No alteration was observed in apigenin (20 mg/kg) treated sham group as compared to sham control group. Postural instability On 14<sup>th</sup> day of treatment there was a significant increase in rotenone treatment group compared to sham control group ( $p < 0.01$ ). This change in postural instability was significantly reversed in apigenin (10 mg/kg and 20 mg/kg) treated group ( $p < 0.05$ ) (Fig.3). There was no significant change in the apigenin (20 mg/kg) as compared to the control group. Rearing behavior. The number of spontaneous rears made during 5 min in the cylinder was measured for each animal. Rotenone treated group consistently reared significantly less compared to vehicle treated animals ( $p < 0.01$ ) at 7<sup>th</sup> and 14<sup>th</sup> day. There was a significant increase in number of rearing counts in apigenin (10 mg/kg and 20 mg/kg) treated group ( $p < 0.05$ ) as compared to the rotenone lesioned group (Fig.4). There was no significant alteration in the apigenin (20 mg/kg) treated group as compared to the control group. Akinesia Rotenone administration caused akinesia (impaired ability to initiate movements) in the treated rats. Rotenone

treatment caused a significant increase in progressive akinesia in 7<sup>th</sup> and 14<sup>th</sup> day ( $p < 0.01$ ). The apigenin (10 mg/kg and 20 mg/kg) treated group showed better performance in the form of significant shorter latency periods ( $p < 0.05$ ) (Fig.5). There was no significant change in the apigenin (20 mg/kg) treated group as compared to the vehicle treated group.

## DISCUSSION

The present study was undertaken to investigate whether apigenin was neuroprotective in unilateral intranigral lesion of rotenone. Rotenone model of PD have been shown to produce symptoms such as dopaminergic degeneration,  $\alpha$ -synucleinopathy and motor dysfunction<sup>3,20,22</sup>. There have been problems with high morbidity and mortality rate associated with systemic rotenone treatment. The advantage of stereotaxic model is its suitability for long-term studies of therapeutic agents. Therefore, an intranigral rotenone lesion rat model of PD was used in this study to determine whether the drug apigenin is neuroprotective to alleviate the symptoms of PD<sup>23</sup>. In assessing motor dysfunction, we used a rotarod test, which revealed a significant reduction in duration of stay in the rotarod after 14 days of treatment with rotenone as compared to the control group. This finding represents an impaired muscular coordination inflicted by rotenone lesioning. Further, apigenin and rotenone cotreated animals showed a significant improvement in muscular coordination and grip strength. On 14<sup>th</sup> day of treatment, there was significant increase in catalepsy and postural instability along with decrease in rearing counts, in rotenone treated group as compared to control group. This change in cataleptic behavior, postural instability and rearing counts due to rotenone treatment was significantly reversed in apigenin (10 mg/kg and 20 mg/kg) treated group as compared to rotenone per se treated group. This shows the therapeutic potential of apigenin against these behavioral symptoms. Flavonoids have been shown to attenuate several behavioral deficits against ischemic reperfusion and neuroinflammatory induced injury. Flavonoids protect neuronal cells from oxidative glutamate toxicity and other forms of oxidative injuries caused by cystine deprivation, BSO, hypoglycemia, and  $H_2O_2$ <sup>24</sup>. This further suggests that apigenin could be useful in preventing the neuronal loss in SNpc and thereby halting the progression of the disease. Conclusion In summary, it has been concluded that unilateral intranigral rotenone lesion induces neuronal damage and dopaminergic neuron loss in substantianigra. Treatment with apigenin abolished the motor dysfunction and renders selective protection against the dying dopaminergic neurons. Thus our results suggest that apigenin may be considered as a potential candidate in PD therapy. Conflict of Interest: Nil

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