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AZELAIC ACID ATTENUATESROTENONE-INDUCED BEHAVIOURAL ALTERATIONS IN PARKINSON'S DISEASE RAT MODEL

Neha Sharma[a,b,c], Navneet Khurana[a], Arunachalam Muthuraman[b,d], and Puneet Utreja[c,e*]
[a]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, PIN- 144 411
[b]Akal College of Pharmacy and Technical Education, GursagarMastuana Sahib, Sangrur, Punjab, India, PIN- 148 001
[c]I.K. Gujral Punjab Technical University, Kapurthala, Punjab, India, PIN- 144 603
[d]Asian Institute of Medicine, Science and Technology, Malaysia
[e]Faculty of Pharmaceutical Sciences, PCTE Group of Institutes, Ludhiana, Punjab, India, PIN- 142 021
*Corresponding author: Dr. Puneet Utreja
Director cum Principal, Faculty of Pharmaceutical Sciences, PCTE Group of Institutes, Ludhiana- 142021, Punjab, India
Tel.: +91-9876827001; E-mail ID:drutreja@gmail.com

ABSTRACT

In the current study, we investigated the anti-Parkinson's effect of azelaic acid (AA; 20 mg/kg, 40mg/kg, 80 mg/kg p.o.) against rotenone (2 mg/kg s.c.) induced Parkinson's disease (PD) rat model. Rotenone was administered continuously for 35 days and caused behavioural alterations such as catalepsy, rigidity in muscles, posture instability and decrease in body weight, locomotor activity, and rearing behaviour.AAshowed significant ($p<0.05$) reversal in behavioural alterations induced by rotenone at 80 mg/kg dose on 28th and 35th day of treatment. Administration of azelaic acid along with levodopa-carbidopa (100mg/kg+25mg/kg p.o.) also showed significant reversal of behavioural alterations induced by rotenone and this effect was comparable to the effect of levodopa-carbidopa treatment group. The study proved the ameliorative effect of azelaic acidin rotenone induced PD rat model indicating its possible therapeutic potential for this devastating disease.

***Keywords*:** Azelaic acid, Oxidative stress, Rotenone, Parkinson's disease

Introduction

Parkinson's disease (PD) is one of the most common progressive neurodegenerative disorder that affects 1% of the population over 60 years of age and is characterized by the symptoms such as rigidity, tremor, akinesia and postural instability (Ogun, 2002; Puspita *et al*., 2017;Wei *et al*., 2018). Current treatments for the disease provide only palliative effect. There is a need for newdrugs that can slow down the course of this disease and prevent or delay the occurrence of PD in susceptible individuals.The pathogenesis of PD includes an appearance of intracytoplasmic inclusions containing α-synuclein and ubiquitin, which is also known as lewy body (Dugger and Dickson, 2017; Spillantini *et al*. 1997) and dopaminergic neuronal degeneration in substantia nigra pars compactaregion. Generation of free radicals is also one of the leading cause of PD which mainly results in cell death due to oxidative stressand reduction in the levels of antioxidants in the brain(Swamy *et al*., 2010; Puspita *et al*., 2017;Hassanzadeh and Rahimmi, 2018;Katerji *et al*., 2019). Thus, antioxidants has shown a potential to be used as an alternative treatment for PD (Sutachan *et al*., 2012; Zhao *et al*., 2019). Azelaic acid (AA) is a dicarboxylic acid found mainly in wheat, rye, and barley. It has been proved for its antioxidant activity (Elewski, 2006) and neuroprotective activity in *in vitro* model (Gan *et al*., 2011a). Thus, in the current study, AA was evaluated for its role in the treatment of PD. The study was designed to evaluate the pharmacological effect of AA in rotenone induced PD model and to evaluate its synergistic effect when administered with levodopa and carbidopa.

Material and Methods

Animals

All the experiments were performed using healthy Sprague Dawley rats (either sex), weighing 200-250 g, between 09:00 and 17:00 h. The animals were procured from CPCSEA approved establishment (Reg no: 1407/a/11/CPCSEA). The animals were housed under standard 12-hour light and dark cycle, with food and water ad libitum. The experiments were performed as per the guidelines of CPCSEA, New Delhi, India.

Chemicals

Rotenone and AA werepurchased from TCI Chemicals, and LobaChemie Pvt. Ltd., India, respectively.

Methodology

Rats were divided into eight groups (n=6). Control group was administered with vehicle which includes sunflower oil (1 ml/kg s.c.) and normal saline (5 ml/kg i.p.). Anothergroup was administered with rotenone at a dose of 2 mg/kg s.c. (emulsified in sunflower oil at 2 mg/ml).Group III, and IV received levodopa-carbidopa (100mg/kg + 25mg/kg p.o.), and AA(80 mg/kg p.o.)respectively. Group V, VI and VII were administered with AA (20, 40, 80 mg/kg

p.o.) along with rotenone. The last group received the combination of AA (80 mg/kg) and levodopa-carbidopa. All the treatments were given continuously for a duration of 35 days and evaluation of behavioural parameters was performed on 1st, 7th, 14th, 21st, 28th, and 35th day of the experiment. Behavioural parameters includes postural instability,rearing behaviour (Woodlee *et al*., 2008; Cannon *et al*., 2009), catalepsy(Verma and Nehru, 2009), muscle activity (Tirumalasetti *et al*., 2015), locomotor activity (Bishnoi *et al*.,2006) and body weight.

Body weight

The body weight of rats was noted down before the start of the experiment and on 1st, 7th, 14th, 21st, 28th, and 35th day.

Catalepsy

Catalepsy, often described as muscle rigidity, was measured in the rats using the bar test. A horizontal bar (9 cm height) was taken and placed on the floor parallely. The animals were placed on the bar in a half rearing position (with the front paws on the bar).The time taken by the rats to remove a paw from the bar was calculated. The cut off of three minutes/180 swas selected for all animals (Costall and Naylor, 1974).

Rearing behaviour

Rats were kept in a clear plexi glass cylinder(height=30 cm; diameter =20 cm) for 5 min. The number of rears (uplifting of the forelimb and touching the wall above shoulder level)done by the rats were recorded manually. It was evaluated by the method given by Cannon *et al*., 2009.

Spontaneous locomotor activity

Locomotor activity was recorded using an actophotometer. This apparatus was comprised of a plastic rodent cage (35 X 35) along with six the infrared photocell beams. Rats were placed one by one in the cage for 10 minutes and cross overs were recorded by the instrument automatically (Habibyar *et al*., 2016).

Postural instability test

Postural instability was determined by holding the rats vertically upside down. The rats were allowed to touch the surface of the table with one forelimb and the displacement distance covered by the rats was recorded. The average of three trials was recorded and reported (Woodlee *et al*., 2008; Cannon *et al*., 2009).

Muscle coordination evaluation

Evaluation of muscle coordination of rats was done by using the standard rotarod apparatus. The apparatus was consisted of rotating rubber rods (1 in. diameter). The speed of the rotation was set at 25 rpm. The cutoff was selected as 2 min for all rats. Latency to fall (in seconds) was counted by the automatic digital counter (Novack and Zwolshen, 1983; Rackham, 1980; Khurana *et al*., 2011).

Statistical analysis

The data was analyzed using two-way ANOVA followed by Tukey test (Sigma Stat Software, 3.5), considering p values <0.05 as statistically significant for all results. The results were expressed as mean ± SEM.

Results

Body weight

Body weight of rotenone treated group was found to be significantly ($P<0.05$) decreased on 28th and 35th day of treatment as compared to the control group. While, there was a significant increase in the body weight of AA treated group(at a dose of 40 and 80 mg/kg) as compared to rotenone treated group on 28th and 35th day of treatment, which was comparable to levodopa carbidopa treated group. However, there was statistically no significant difference was found between levodopa carbidopa treated group and group which received the combination of AA (80 mg/kg) and levodopa carbidopa (Fig. 1).

Catalepsy

There was a statistically significant ($P<0.05$) increase in the catalepsy in rotenone treated group as compared to control group on 14th, 21st, 28th, and 35th day of treatment. While, it was found that there was a significant reduction in catalepsy of AA treated group (at a dose of 40 and 80 mg/kg)on 28th and 35th day of treatment as compared to rotenone treated group. However, no significant difference was observed between levodopa carbidopa treated group and group which received the combination of AA (80 mg/kg) and levodopa carbidopa **(Fig. 2**).

Rearing behaviour

In this test, statistically significant ($P<0.05$) decrease in the number of rears was found in the rotenone treated group as compared to control group. But, significant increase in the number of rears was observed in AA (40 and 80 mg/kg) treated group on 21st, 28th and 35th day as compared to rotenone treated group. However, there was statistically no significant difference between levodopa carbidopa treated group and group which received the combination of AA (80 mg/kg) and levodopa carbidopa **(Fig. 3**).

Spontaneous locomotor activity

Locomotor activity of rotenone treated group was found to be decreased statistically significant ($P<0.05$) as compared to the control group. There was significant rise in locomotion activity of AA (80 mg/kg) treated groups on 35th day of treatment as compared to rotenone treated group. While, there was no significant difference observed between levodopa carbidopa treated group and group which received the combination of AA (80 mg/kg) and levodopa carbidopa **(Fig. 4**).

Postural Instability test

There was statistically significant ($P<0.05$) rise in the postural instability of rotenone treated group on 7th, 14th, 21st, 28th, and 35th day of experiment as compared to control group. There was significant reduction in the postural instability of AA (40 and 80 mg/kg) treated group on 28th and 35th day of treatment as compared to rotenone treated group. But, no significant difference between levodopa carbidopa treated group and group which received the combination of AA (80 mg/kg) and levodopa carbidopa **(Fig. 5**).

Muscle coordination evaluation

Statistically significant ($P<0.05$) decrease in the muscle strength of rotenone treated group was recorded as compared to control group. Significant rise in the muscle coordination was found inAA (80 mg/kg) treated group on 35th day of treatment as compared to rotenone treated group. However, there was no significant difference between levodopa carbidopa treated group and group which received the combination of AA (80 mg/kg) and levodopa carbidopa **(Fig. 6**).

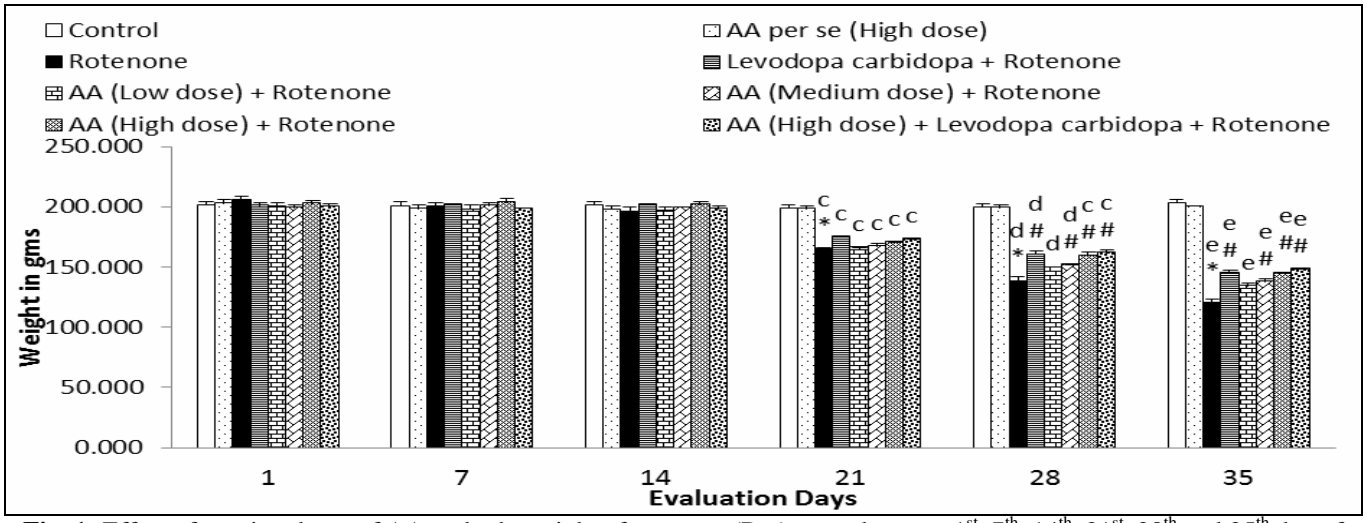

Fig. 1: Effect of varying doses of AA on body weight of rotenone (Rot) treated rats on 1st, 7th, 14th, 21st, 28th and 35th day of treatment. * represent significant difference between group 1 and 3. # represents significant difference between groups 4, 5, 6, 7, 8 with group 3, a, b, c, d, e represents significant difference vs. day 0, 7, 14, 21 and 28, respectively; data represented as mean ± S.E.M (n=6).

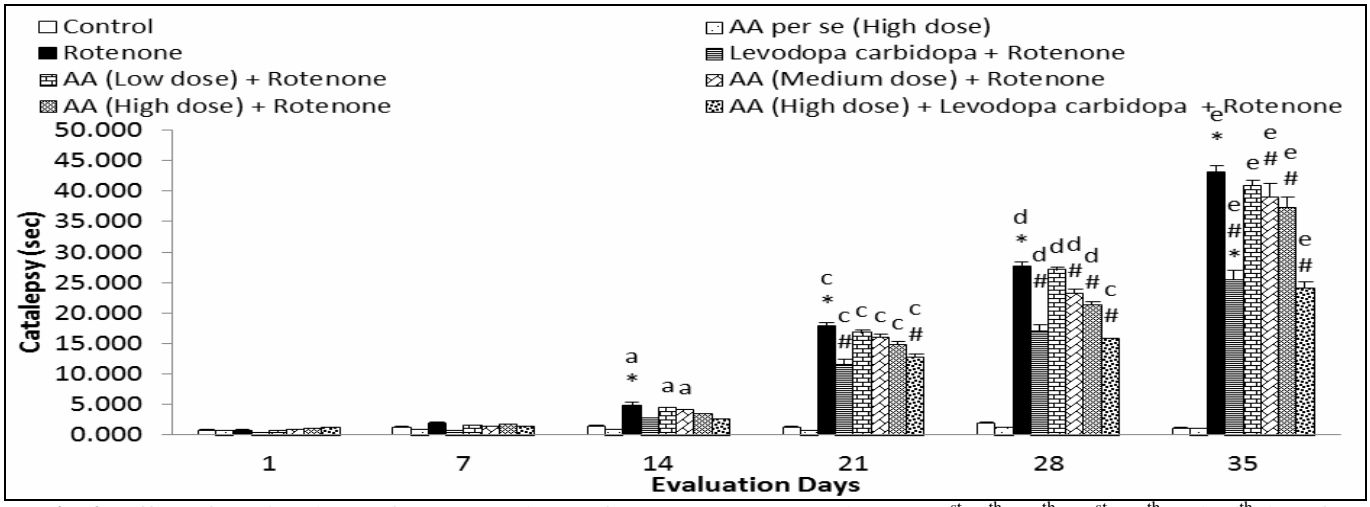

Fig. 2: Effect of varying doses of AA on catalepsy of rotenone (Rot) treated rats on 1st, 7th, 14th, 21st, 28th and 35th day of treatment. * represent significant difference between group 1 and 3. # represents significant difference between groups 4, 5, 6, 7, 8 with group 3, a, b, c, d, e represents significant difference vs. day 0, 7, 14, 21 and 28, respectively; data represented as mean ± S.E.M (n=6).

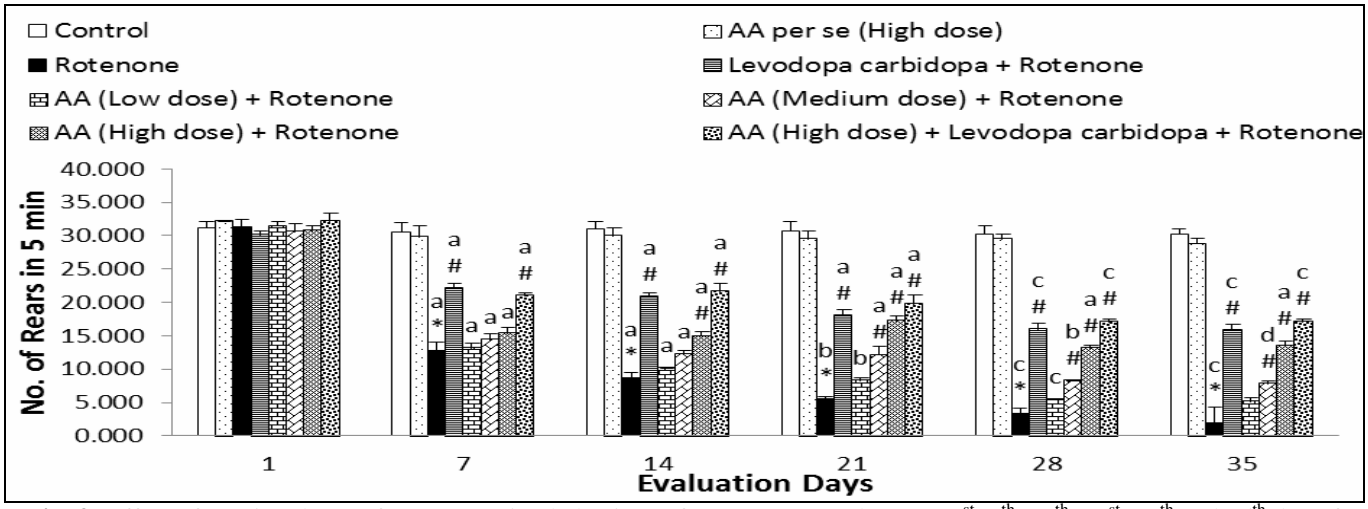

Fig. 3: Effect of varying doses of AA on rearing behaviour of rotenone treated rats on 1st, 7th, 14th, 21st, 28th and 35th day of treatment. * represent significant difference between group 1 and 3. # represents significant difference between groups 4, 5, 6, 7, 8 with group 3, a, b, c, d, e represents significant difference vs. day 0, 7, 14, 21 and 28, respectively; data represented as mean ± S.E.M (n=6).

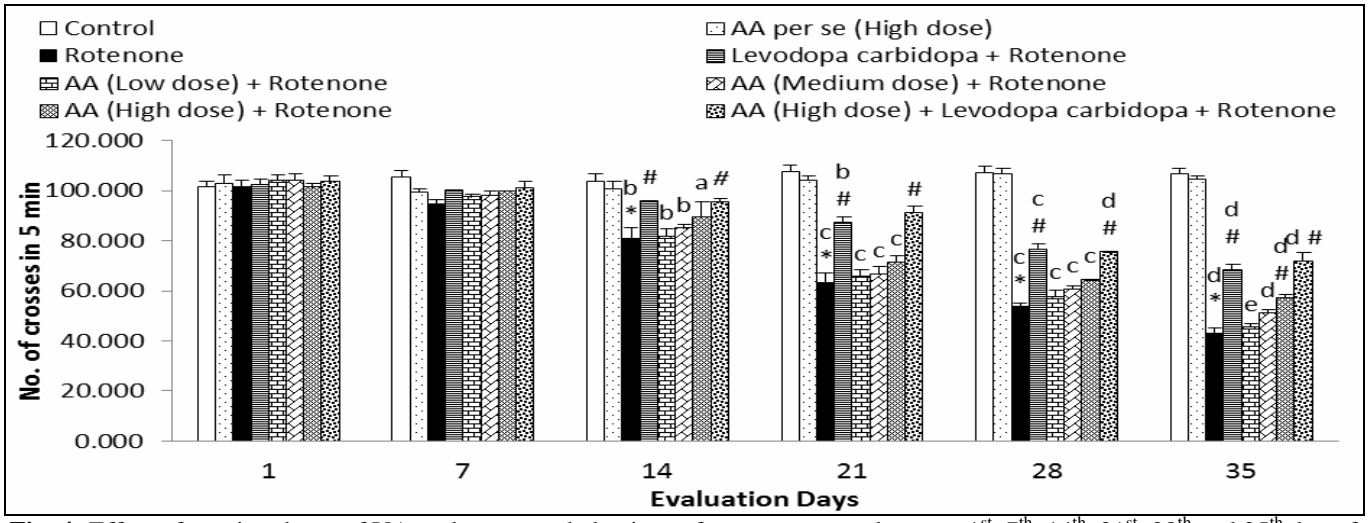

Fig. 4: Effect of varying doses of VA on locomotor behaviour of rotenone treated rats on 1st, 7th, 14th, 21st, 28th and 35th day of treatment. * represent significant difference between group 1 and 3. # represents significant difference between groups 4, 5, 6, 7, 8 with group 3, a, b, c, d, e represents significant difference vs. day 0, 7, 14, 21 and 28, respectively; data represented as mean ± S.E.M (n=6)

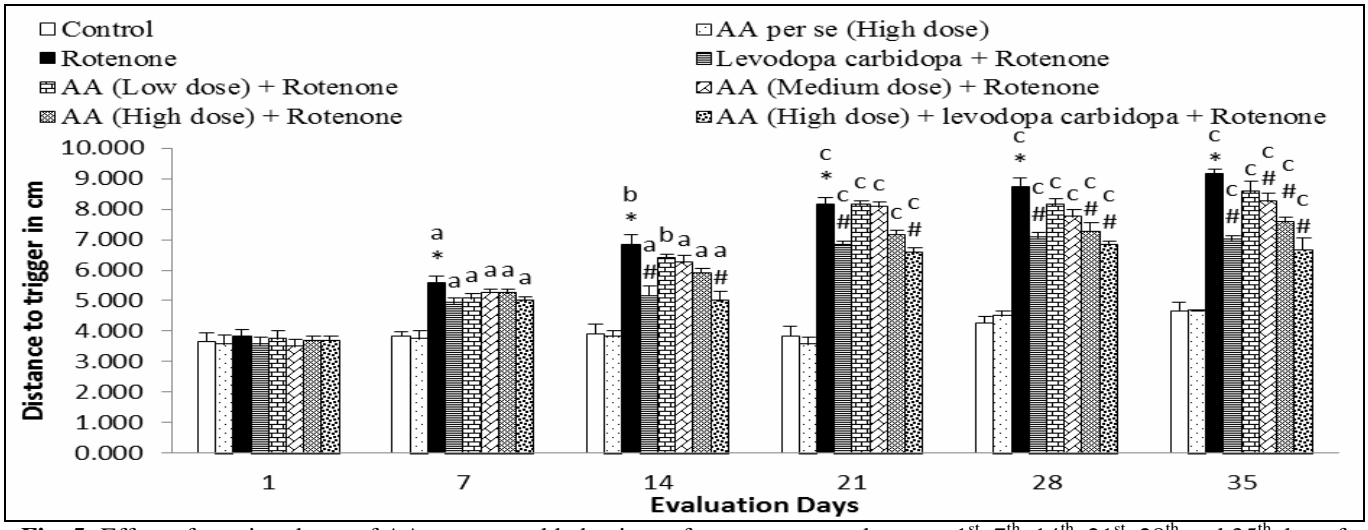

Fig. 5: Effect of varying doses of AA on postural behaviour of rotenone treated rats on 1st, 7th, 14th, 21st, 28th and 35th day of treatment. * represent significant difference between group 1 and 3. # represents significant difference between groups 4, 5, 6, 7, 8 with group 3, a, b, c, d, e represents significant difference vs. day 0, 7, 14, 21 and 28, respectively; data represented as mean ± S.E.M (n=6).

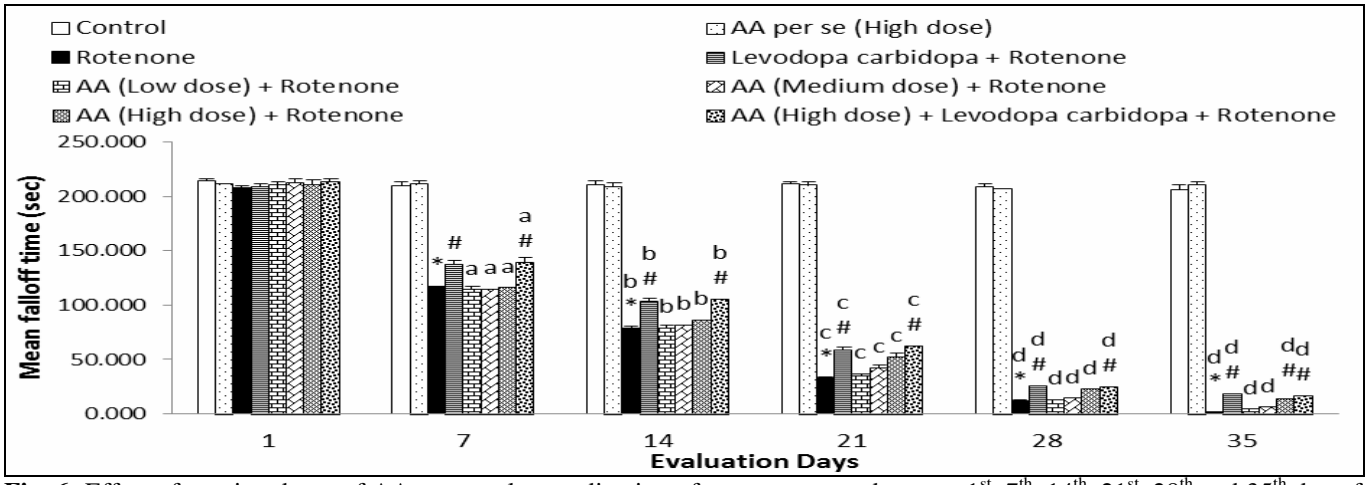

Fig. 6: Effect of varying doses of AA on muscle coordination of rotenone treated rats on 1st, 7th, 14th, 21st, 28th and 35th day of treatment. * represent significant difference between group 1 and 3. # represents significant difference between groups 4, 5, 6, 7, 8 with group 3, a, b, c, d, e represents significant difference vs. day 0, 7, 14, 21 and 28, respectively; data represented as mean ± S.E.M (n=6).

Discussion

The present study was designed for the evaluation of neuroprotective effect of AA in rotenone induced PD model and to evaluate its synergistic effect with standard drugs. In this experiment, AA was evaluated against the behavioral changes induced by the rotenone in rats. AA has been proven for its antioxidant activity in skin disorder and neuroprotective effect in *in vitro* experiment (Elewski, 2006; Gan *et al*., 2011).

Levodopa and carbidopa are the standard drugs which mainly increase the level of dopamine in brain in PD patients(Salat and Tolosa, 2013). In the current study, AA administered at high dose significantly attenuated the rotenone-induced changes in body weight, catalepsy, locomotor activity, postural instability, rearing behaviour and muscle co-ordination. All effects were significant at high dose (80 mg/kg) of AA, but the group who received combination of AA, levodopa and carbidopa has not shown any synergistic effect. The result of this group was comparable to the group who received standard drug.

Thus, the AA has shown significant effect on behavioural parameters. The exact mechanism responsible for the effectiveness of AA can be hypothesized due to its antioxidant effect.

Conclusion

It can be concluded that AA caused the reversal of all the behavioural changes in the rats induced by rotenone. These effects of AA can be due to its antioxidant ability in rats' brain. AA at 80 mg/kg showed the significant effect in all the parameters. Hence, it can be concluded that AA can be used as a potential treatment for the PD.

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