

Rotarod test and Catalepsy bar test: behavioral testing and neuromodulation of *Aloe vera* in MPTP induced Parkinson's disease animal model

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ABSTRACT

Background: *Aloe vera* (Family Liliaceae) has been used for the treatment of diabetes, skin disorders and as anti-inflammatory agent. Objectives: behavioral testing of antiparkinsonian effect of *Aloe vera* in MPTP induced animal model.

Methods: Rotarod test and Catalepsy bar test were used for behavioral assessment. Assessment of oxidative stress was done in the striatal region of the brain by reduced glutathione (GSH) measurement.

Results: *A. vera* (200 and 400mg/kg, p.o.) was found to significantly increase the retention time in rota rod test and significantly decrease the latency period in catalepsy bar test as compared to MPTP groups. *A. vera* was found to have significant anti-oxidative effect in the striatal region of the brain by GSH measurement.

Conclusions: Thus, it can be proposed that *A. vera* has a potential anti-parkinson effect in mice.

Keywords: *Aloe vera*, Anti-oxidant, GSH, MPTP

INTRODUCTION

Since ancient times, plants have been used to treat various diseases and have been an exemplary source of medicine.¹ *Aloe vera* (Family: Liliaceae), is one such ancient plant whose medicinal properties have been known since centuries.² It has been found effective in improving lipid profile status in rats with streptozotocin-induced diabetes.³

In addition, recent studies reveal the role of *A. vera* in immunomodulation, inflammatory pain, anti-depressant and memory enhancing properties.^{4,5}

A recent study has reported that *A. vera* improves antioxidant activity within the hippocampus and cerebral cortex leading to improvement of the motor and memory behavioral tasks in diabetic mice.⁶ Such report suggests that *A. vera* might have some beneficial effects in the treatment of some central nervous system diseases.

The clinical syndrome of PD results from idiopathic degeneration of the dopaminergic cells in the pars compacta of the substantia nigra.⁷ While the cause of the degeneration of the dopaminergic cells in the pars

compacta of the substantia nigra is not known, oxidative stress plays an important role.⁸

Among different pharmacological treatments, levodopa remains the most efficacious and is still the mainstay of therapy. However, long-term use of levodopa can cause disabling motor complications, particularly dyskinesias and motor fluctuations, which limit its usefulness. Because of the concern about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment has been on the rise in the last few decades. Thus, strategies employing antioxidant and neuroprotective from natural sources can be a good approach in improving the treatment of Parkinson's disease.

So, efforts have been made in the present study to explore the effects of *A. vera* on animal models of Parkinson's disease by investigating its effect on behavioral models, oxidative stress changes induced by MPTP in mice.

METHODS

Animals

Swiss albino mice (6 weeks old) of either sex weighing between 25 and 30gms were used for the study. The study was duly approved by the Institutional Animal Ethics Committee. The animals were housed in polypropylene cages in groups of six to eight mice per cage and kept under controlled environmental condition (temperature 22±2°C, humidity 50-55%, natural light/day cycle). All the experiments were performed at daytime between 09:30 and 15:30 hours. Care of animals was according to the guidelines of (CPSCEA) Committee for the Purpose of Control and Supervision of Experiments on Animals.

Plant material

A. vera extract was obtained from M/s Indo World Trading Corporation, New Delhi (Batch no. IWTC/711/9432). As per the literature provided by the manufacturer, the gel obtained from *Aloe vera* leaf was mixed with double distilled water in the ratio 1:1, mechanically shaken at room temperature and concentrated in the evaporator, followed by lyophilisation to obtain a brown powder with characteristic odour. The characterization of a sample of the extract by the spectrophotometer (IP66 method) revealed 3.14% aloin. For the purpose of study, the *A. vera* powder was dissolved in double distilled water to prepare suspensions of required doses of 100, 200 and 400mg/kg.

Experimental design

The animals were divided into 11 groups (n =12).

- Group I- was chronically administered distilled water (orally, once per day ×15 days).

- Group II- received MPTP (2 doses, each dose 20 mg/kg at 2 hr. interval, i.p. daily × 15 days).
- Groups III, IV, V- were chronically treated with *A. vera* (100, 200 and 400mg/kg/day, orally), respectively, x 15 days along with MPTP.
- Group VI- received Levodopa (30mg/kg, i.p, once per day x15 days) along with MPTP.

The *A. vera* (100, 200mg/kg, 400mg/kg) orally and Levodopa (30mg/kg, i.p.) were given 30 minutes prior to injections of first dose of MPTP for 15 days of experimental period. MPTP (salt) and Levodopa were obtained from Sigma Chemical Co. USA and all other chemicals used were of analytical grade.

Assessment of behavioral tests

Rota rod test

The rota rod method was used similar to the one described by Dunham and Miya.⁹ The speed selector was set so that the roller rod make 15 rpm. Prior to the test, each animal was given 1minute exposure to the moving rod. The animals were placed on the roller for 3 minutes. Latency to fall from rolling rod was observed. A normal animal could maintain its equilibrium for an indefinite period of time. Movement impairment was indicated by the inability of the animal to remain on the roller for a 300 minute test period.⁹

Catalepsy bar test

The test was performed by the method as described by Hoffman et al.¹⁰ Catalepsy was measured by means of a standard bar test, as the time that animal maintained an imposed position with both front limbs raised and resting on a three centimeter high wooden bar (0.9cm in diameter). The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. Catalepsy was induced with MPTP. A cut off time of 720 seconds was applied. Catalepsy was assessed by standard bar test on the 7th day and on the 15th day.

At the end of 15 days of experimental period, the animals were sacrificed using ether anesthesia and brains were taken out for assessment of oxidative stress changes.

Assessment of oxidative stress

Assessment of oxidative stress was done in the striatal region of the brain by reduced glutathione (GSH) in 6 mice of each group.

Estimation of Reduced Glutathione (GSH)

Reduced glutathione was estimated by the method described by Ellman.¹¹ This method is based on the development of a yellow colour when 5,5'-dithio-bis-2-

nitrobenzoic acid (DTNB) is added to compounds containing sulfhydryl groups.

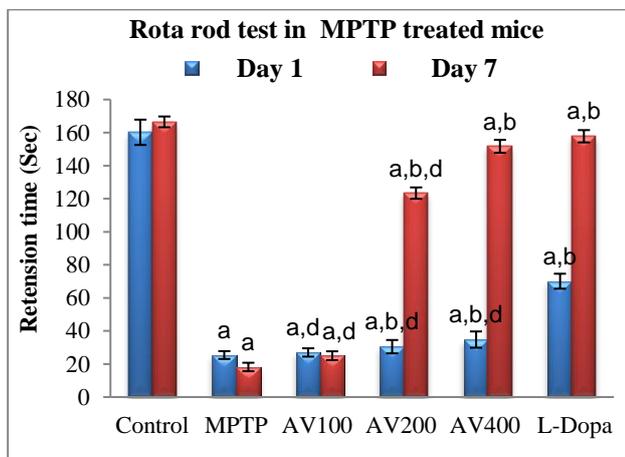
Statistical analysis

Results of the above experiments were expressed as Mean±SEM, and the difference between means was analysed by analysis of variance (ANOVA) using graph pad prism followed by post-hoc Tukey test, with P <0.05 being considered as statistical significant.

RESULTS

Rota rod test

Among MPTP alone treated groups, significant decrease in retention time (p<0.001) was seen on day ‘7’ and day ‘15’ as compared to control group. In Levodopa treated group, significant increase in retention time (p<0.001) was seen on day ‘7’ and day ‘15’ as compared to MPTP treated groups. However, unlike Levodopa treated group, *A. vera* 100,200mg/kg and 400mg/kg pretreated groups did not cause any significant change in retention time on day ‘7’. But on day ‘15’ *A. vera* 200mg/kg and 400mg/kg groups showed significant increase in retention time (p<0.001) when compared to MPTP (Figure 1), whereas no significant difference in retention time was seen when compared to levodopa treated group.



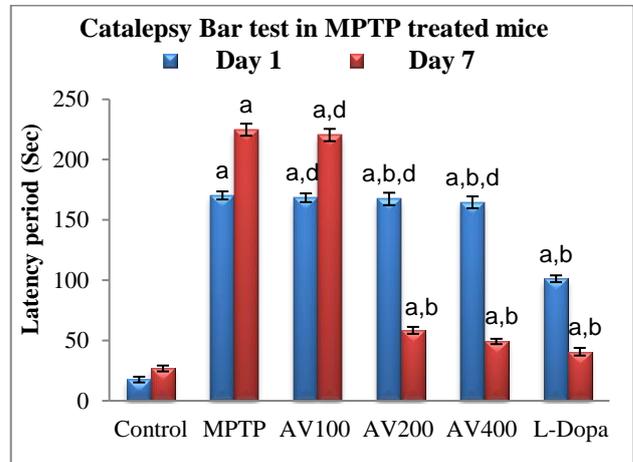
^ap<0.001 as compared to control (distilled water) group
^bp<0.001 as compared to MPTP group
^dp<0.001 as compared to Levodopa + MPTP group
 Values are expressed as Mean±SEM

Figure 1: Effect of *A. vera* (AV) on Rota rod test in MPTP treated mice (n=12).

Catalepsy bar test

Among MPTP alone treated groups, significant increase in latency period (p<0.001) was seen on day ‘7’ and day ‘15’ as compared to control group. In Levodopa treated group, significant decrease in latency period (p<0.001) on day ‘7’ and day ‘15’ was seen as compared to MPTP treated groups. However, unlike Levodopa treated group, *A. vera*

100, 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in latency period on day ‘7’. But on day ‘15’ *A. vera* 200mg/kg and 400mg/kg groups showed significant decrease in latency period (p<0.001) when compared to MPTP treated groups (Figure 2) whereas no significant difference in latency period was seen when compared to levodopa treated group.

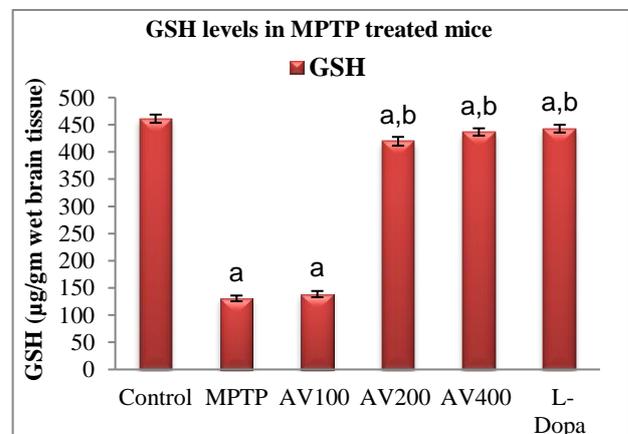


^ap<0.001 as compared to control (distilled water) group.
^bp<0.001 as compared to MPTP group
^dp<0.001 as compared to Levodopa + MPTP group
 Values are expressed as Mean±SEM.

Figure 2: Effect of *A. vera* (AV) on Catatonic response in MPTP treated mice (n=12).

Estimation of reduced glutathione

Among MPTP treated groups, significant increase in brain significant decrease in brain GSH levels (p<0.001), was seen as compared to control group. *A. vera* 200mg/kg, 400mg/kg and Levodopa pretreated groups showed significant increase (p<0.001) in brain GSH levels when compared to MPTP treated groups (as shown in Figure 3). *Aloe vera* 200 and 400mg/kg treated groups did not show significant difference in brain GSH levels when compared to Levodopa treated group.



^ap<0.001 as compared to control (distilled water) group.
^bp<0.001 as compared to MPTP group
 Values are expressed as Mean±SEM.

Figure 3: Effect of *A. vera* (AV) on brain levels of GSH in MPTP treated mice (n=6).

DISCUSSION

Parkinson's disease is one of the most common neurodegenerative disorders, characterized by degeneration of dopamine producing neurons in the substantia nigra and released in the caudate nucleus and putamen leading to resting tremor, bradykinesia, shuffling gait, flexed posture and rigidity.

While the cause of the degeneration is not known, oxidative stress plays an important role.⁸ Oxidative stress may arise from the metabolism of dopamine with the production of potentially harmful free radical species.¹² Compared to the rest of brain, the substantia nigra pars compacta is exposed to a higher rate of ROS formation and to higher levels of oxidative stress. This may be related to the energy metabolism of these cells or to their high content of dopamine.¹³ Various studies have reported oxidative stress changes in the brain of Parkinson's disease patients.¹⁴

Neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are used commonly to create experimental model of Parkinson's disease by which certain aspects of the disease such as catalepsy, motor imbalance and slowing of movement can be studied. MPTP is a highly lipophilic molecule, after systemic absorption it crosses the blood brain barrier immediately. Within the brain, MPTP is rapidly converted to the hydrophilic metabolite 1-methyl-4 phenylpyridinium ion (MPP⁺) and is responsible for the dopaminergic neuron loss. The involvement of these free radicals play major role in the pathogenesis of this movement disorder.¹⁵ Neuroleptics like haloperidol induced catalepsy has been linked to a blocking of post synaptic striatal dopamine D1, D2 receptors and studies have proposed reactive oxygen species as cause of haloperidol induced toxicity.¹⁶ Drugs which attenuate haloperidol-induced motor disorders might reduce the extrapyramidal signs of Parkinson's disease. The two behavioral parameters - Rota rod performance and Catatonic response were measured as Retention time (sec) and Latency period (sec) respectively.

In MPTP treated groups, 15 days treatment with *A. vera* (200, 400mg/kg, p.o.), significantly increased the retention time (sec) in rota rod, decreased the latency period (sec) in catalepsy model and this effect is comparable to that of levodopa group. The above findings of behavioral tests are similar with other previous studies.^{17,18}

The assessment of biochemical parameters of oxidative stress were done by measuring brain reduced glutathione (GSH) levels. MPTP treated groups showed significant decrease in GSH levels. *A. vera* (200, 400mg/kg) and levodopa caused significant increase in brain GSH levels. Our results of biochemical tests are in accordance with

previous studies.¹⁹ Thus, the oxidative stress parameters (GSH) are also positively modulated by *A. vera* so as to decrease the oxidative damage to neurons.

A. vera is an important medicinal plant that plays a significant role in protection from oxidative stress. A number of studies have shown that *A. vera* has significant anti-oxidant properties.²⁰ It has been hypothesized that antioxidants may be neuroprotective in PD, by preventing neuronal death caused by intracellular free radicals.⁸

Inquiries into the role of neuroinflammation in Parkinson's disease have coincided with increasing interests in determining whether anti-inflammatory medications may be helpful in preventing PD. Experimental evidence and animal models in particular support a preventative role for nonsteroidal anti-inflammatory drugs (NSAIDs) in Parkinson's disease. For example, studies have demonstrated that anti-inflammatory drugs such as acetylsalicylic acid are protective against MPTP-induced striatal dopamine depletion in mice.²¹ Recently, involvement of inflammatory process has been also reported in the pathogenesis of Parkinson's disease.^{14,21} It is widely accepted that inflammation and oxidative stress are interrelated. Oxidative stress can increase inflammatory activity and, conversely, inflammation is known to cause oxidative stress.²²

Several studies have also emphasized the anti-inflammatory properties of *Aloe vera* in mice and rats. Previous studies show that *Aloe vera* leaf gel extract was found to have anti-inflammatory, memory enhancing and anti-depressant properties.^{4,5,23,24}

A. vera leaf gel is known to be rich in anthraquinones such as aloe-emodin, aloetic acid, anthranol, aloin A and B. Aloin is known to exert anti-inflammatory activity in the rat colitis, and the present extract of *A. vera* contains relatively high amount (3.14%) of aloin. Further studies are needed to prove whether anti-inflammatory and anti-oxidant properties of aloin is responsible for the anti-parkinson effect or whether the synergy of a number of components viz. barbaloin, glucomannan, acemannan, minerals, flavonoids, tannic acid, etc. is responsible for the observed effects.

It can be proposed that apart from the known effects of *A. vera*, it also has neuro-protective and anti-oxidant properties and thus further studies are required to clearly establish its role as an anti-parkinson agent.

CONCLUSION

Parkinson's disease is a progressive neurodegenerative disease accompanied by preferential loss of dopaminergic neurons of the substantia nigra pars compacta. Neurotoxins such as MPTP is commonly used to create experimental model of Parkinson's disease. The potent Parkinsonian neurotoxin MPTP has been shown to

cause dopaminergic neurodegeneration by generation of free radicals leading to oxidative stress as shown by alteration in the states of antioxidant enzymes and molecules. Oxidative stress changes are seen in the brain of Parkinson's disease patients.

The results of the present study conclusively showed that *A. vera* has antioxidant activity and neuroprotective role in MPTP experimental models of Parkinson's disease. *A. vera* found to be effective in increasing Rota rod performance and decreasing Catatonic response. The neuro-modulatory effect of *A. vera* on behavioral, oxidative stress changes may be due its neuroprotective, anti-oxidant properties. In this regard, future studies on this topic may provide an elaborate view to use *A. vera* in clinical medicine for treatment of Parkinson's disease and its neurological sequel.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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