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EFFECT OF ALOE VERA ON ANIMAL MODELS OF PARKINSON DISEASE IN MICE

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ABSTRACT

Aloe vera (Family: Liliaceae) has been used for the treatment of diabetes, skin disorders and as anti-inflammatory agent. To evaluate the anti-parkinson effect, Rota rod and catalepsy bar models were used. Assessment of oxidative stress was done in the striatal region of the brain by malondialdehyde (MDA) and reduced glutathione (GSH) measurement. A.vera (200 and 400 mg/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 and haloperidol groups. A.vera was found to have significant anti-oxidative effect in the striatal region of the brain by MDA and GSH measurement. Histopathological analysis of brain tissue of A.vera treated groups revealed minimal neuronal destruction as compared to MPTP and haloperidol groups. Thus it can be proposed that A.vera has a potential anti-parkinson effect in mice.

KEYWORDS: Aloe vera, Anti-oxidant, MPTP, Haloperidol, MDA, GSH
INTRODUCTION

Since ancient times, plants have been used to treat various diseases and have been an exemplary source of medicine\(^1\). \textit{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 \textit{A. vera} in immunomodulation, inflammatory pain, antidepressant and memory enhancing properties\(^4,5\). A recent study has reported that \textit{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 \textit{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\(^8\). Although Levodopa is the most potent drug to treat Parkinson’s disease patients, its long term usage results in complications like dyskinesias, on-off phenomenon. 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 \textit{A. vera} on animal models of Parkinson’s disease. Hence, to show the effect of \textit{A. vera} on behavioral parameters, oxidative stress, histological changes produced by MPTP and haloperidol in mice is carried out.

MATERIALS AND METHODS

Animals

Swiss albino mice (6 weeks old) of either sex weighing between 25 and 30 g, obtained from the Central Animal House of University College of Medical Sciences and Guru Teg Bahadur Hospital, were used. 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 the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forest, Government of India, New Delhi. The study was duly approved by the Institutional Animal Ethics Committee, University College of Medical Sciences, Delhi. (Approval No. IAEC/2011/49 dated 10 March 2011).

Plant Material

\textit{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 \textit{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 characterisation of a sample of the extract by the spectrophotometer (IP66 method) revealed 3.14 % aloin. For the purpose of study, the \textit{A. vera} powder was dissolved in double distilled water to prepare suspensions of required doses of 100, 200 and 400 mg/kg.

Experimental Design

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

- **Group I** - was chronically administered distilled water (orally, once per day \(\times 1\) weeks).
- **Group II** - received MPTP (2 doses, each dose 20 mg/kg at 2 hr. interval, i.p. daily \(\times 1\) week).
- **Groups III, IV, and V** - were chronically treated with \textit{A. vera} (100, 200, and 400 mg/kg/day,
orally), respectively, x 1 week along with MPTP.
**Group VI**- received Levodopa (30mg/kg, i.p, once per day x 1 week) along with MPTP.
**Group VII**- received Haloperidol (1mg/kg, i.p. once per day x 1 week).
**Group VIII, IX and X**- were chronically treated with A.vera (100, 200, and 400 mg/kg/day) orally, respectively, x1 weeks along with Haloperidol.
**Group XI**- received Levodopa (30mg/kg, i.p. once per day x 1 week) along with Haloperidol.

The A.vera (100mg/kg, 200mg/kg, 400mg/kg, orally) and Levodopa (30mg/kg, i.p.) were given 30 minutes prior to injections of first dose of MPTP and haloperidol for 7 days of experimental period. MPTP (salt) and Haloperidol, Levodopa were obtained from Sigma Chemical Co. USA and all other chemicals used were of analytical grade.

**ASSESSMENT OF BEHAVIORAL TESTS**

1. **Rota rod test**
   The rota rod method was used similar to the one described by Dunham and Miya. The speed selector was set so that the roller rod would make 15 rpm. Prior to the test, each animal was given 1 minute 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 3 minute test period.

2. **Catalepsy bar test**
   The test was performed by the method as described by Hoffman et al. Catalepsy was measured by means of a standard bar test, as the time that animal maintained an imposed position with both front paws raised and resting on wooden bar (diameter 0.7 cm), 9 cm above the surface. The cataleptic period was estimated by length of time the animal remains in an imposed position. The end 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 by MPTP and haloperidol. Latency period at different time point intervals (0, 60, 120, 180, 240 minutes) after MPTP and haloperidol administration were added and expressed as average latency period. A cut off time of 180 seconds was applied. Catalepsy was assessed by standard bar test on the first day and on the seventh day. At the end of 7 days of experimental period, the animals were sacrificed using ether anesthesia and brains were taken out for assessment of oxidative stress changes and histological analysis.

**ASSESSMENT OF OXIDATIVE STRESS**

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

**Estimation of Malondialdehyde (MDA)**
Malondialdehyde (indicator of lipid peroxidation) was estimated as described by Okhawa et al. MDA reacts with thiobarbituric acid (TBA), under acidic conditions on heating the solution at 95°C pink colour is formed. The absorbance of colour is assessed by spectrophotometer at 535 nm.

**Estimation of Reduced Glutathione (GSH)**
Reduced glutathione was estimated by the method described by Ellman. 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.

**HISTOPATHOLOGICAL ANALYSIS**
The brain specimens obtained from the rest of the 6 mice from each group of animals were fixed in 10% formalin. The tissue blocks from brain specimen were processed and embedded in paraffin wax. 4-5 µm thickness section was cut and stained with haematoxylin- eosin. Histologic changes were observed under light microscope.

**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**

groups, significant decrease in retention time \( (p<0.001) \) was seen on day ‘0’ and day ‘07’ as compared to control group. In Levodopa treated group, significant increase in retention time \( (p<0.001) \) was seen on day ‘0’ and day ‘07’ as compared to MPTP and haloperidol treated groups. However, unlike Among MPTP and haloperidol alone treated

Levodopa treated group, **A. vera** 100mg/kg, 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in retention time on day ‘0’. But on day ‘7’ **A. vera** 200 mg/kg and 400mg/kg groups showed significant increase in retention time \( (p<0.001) \) when compared to MPTP (as shown in Figure 1) and haloperidol treated groups (as shown in Figure 2), whereas no significant difference in retention time was seen when compared to levodopa treated group.

**Figure 1**

*Effect of A. vera (AV) on Rota rod test in MPTP treated mice (n=12). Values are expressed as Mean ± SEM.*

**Figure 2**

*Effect of A. vera (AV) on Rota rod test in Haloperidol treated mice (n=12). Values are expressed as Mean ± SEM.*
Catalepsy bar test
Among MPTP and haloperidol alone treated groups, significant increase in the latency period (p<0.001) was seen on day ‘0’ and day ‘07’ as compared to control group. In Levodopa treated group, significant decrease in the latency period (p<0.001) on day ‘0’ and day ‘07’ was seen as compared to MPTP and haloperidol treated groups. However, unlike Levodopa treated group, A. vera 100mg/kg, 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in the latency period on day ‘0’. But on day ‘7’ A. vera 200mg/kg and 400mg/kg groups showed significant decrease in latency period (p<0.001) when compared to MPTP treated groups (as shown in Figure 3) and haloperidol treated groups (as shown in Figure 4), whereas no significant difference in latency period was seen when compared to levodopa treated group.

Figure 3
Effect of A. vera (AV) on Catatonic response in MPTP treated mice (n=12).
Values are expressed as Mean ± SEM.

Figure 4
Effect of A. vera (AV) on Catatonic response in Haloperidol treated mice (n=12).
Values are expressed as Mean ± SEM.
Estimation of Malondialdehyde (MDA) and reduced Glutathione
Among MPTP and haloperidol treated groups, significant increase in brain MDA levels (p<0.001) and 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 decrease (p<0.001) in brain MDA levels and significant increase (p<0.001) in brain GSH levels when compared to MPTP treated groups (as shown in Figure 5) and haloperidol treated groups (as shown in Figure 6). *A. vera* 200 and 400mg/kg treated groups did not show significant difference in brain MDA and GSH levels when compared to Levodopa treated group.

Figure 5
Effect of *A. vera* (AV) on brain levels of MDA & GSH in MPTP treated mice (n=6).
Values are expressed as Mean ± SEM.

Figure 6
Effect of *A. vera* (AV) on brain levels of MDA & GSH in Haloperidol treated mice (n=6).
Values are expressed as Mean ± SEM.

Histopathological Analysis of mice Brain
Figure 7. Normal architecture of mice brain in Control group.
Photomicrograph showing normal architecture of striatal region of mice brain with darkly stained neurons in control group (H&E stain x 40).
Figure 8. MPTP treated group.
Photomicrograph showing striatal area of mice brain in MPTP treated group in which neuronal hypertrophic changes accompanied with Karyorrhexis (indicated by Arrow) and significant decrease in neurons as compared to control group is seen (H&E x 40).

Figure 9. A. vera (100 mg/kg, p.o.) + MPTP treated group.
Photomicrograph showing architecture of striatal area of mice brain in A. vera (100 mg/kg, p.o.) + MPTP treated group, in which moderate decrease in neurons along with moderate cellular hypertrophy and mild Karyorrhexis is seen (H&E x 40).

Figure 10. A. vera (200 mg/kg, p.o.) + MPTP treated group.
Photomicrograph showing striatal area of mice brain in A. vera (200 mg/kg, p.o.) + MPTP treated group in which mild decrease in neurons and very few cells showing hypertrophy and moderate Karyorrhexis are seen (H&E x 40).
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. Oxidative stress may arise from the metabolism of dopamine with the production of potentially harmful free radical species. 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. Various studies have reported oxidative stress changes in the brain of...
Parkinson’s disease patients\textsuperscript{16}. Neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and drugs which block dopamine receptors like haloperidol 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\textsuperscript{+}) and is responsible for the dopaminergic neuron loss. The involvement of these free radicals play major role in the pathogenesis of this movement disorder\textsuperscript{17}. Neuroleptics like haloperidol induced catalepsy has been linked to a blocking of post synaptic striatal dopamine D2 receptors and studies have proposed reactive oxygen species as cause of haloperidol induced toxicity\textsuperscript{18}. 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 and Haloperidol treated groups, 07 days treatment with \textit{A. vera} (200, 400 mg/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\textsuperscript{19,20}. The assessment of biochemical parameters of oxidative stress was done by measuring brain malondialdehyde (MDA) and reduced glutathione (GSH) levels. MPTP and haloperidol treated groups showed significant increase in brain MDA and decrease in GSH levels. \textit{A. vera} (200, 400mg/kg) and levodopa caused significant decrease in brain MDA and the increase in brain GSH levels. Our results of biochemical tests are in accordance with previous studies\textsuperscript{21}. Thus, the oxidative stress parameters (MDA and GSH) are also positively modulated by \textit{A. vera} so as to decrease the oxidative damage to neurons. In our present study, histologic findings indicates that MPTP and haloperidol treated groups showed moderate decrease in neurons, with moderate cellular hypertrophy and Karyorrhexis when compared to control group. Pretreatment of \textit{A. vera} particularly 200 and 400mg/kg significantly prevented these neuronal changes from occurring there by confirming its neuroprotective effect. \textit{A. vera} is an important medicinal plant that plays a significant role in protection from oxidative stress. A number of studies have shown that \textit{A. vera} has significant anti-oxidant properties\textsuperscript{22}. It has been hypothesized that antioxidants may be neuroprotective in PD, by preventing neuronal death caused by intracellular free radicals\textsuperscript{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\textsuperscript{23}. Recently, involvement of inflammatory process has been also reported in the pathogenesis of Parkinson’s disease\textsuperscript{15,23}. 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\textsuperscript{24}. Several studies have also emphasized the anti-inflammatory properties of \textit{Aloe vera} in mice and rats. Previous studies shows that \textit{Aloe vera} leaf gel extract was found to have anti-inflammatory property\textsuperscript{4,5,25}. \textit{A. vera} leaf gel is known to be rich in anthraquinones such as aloemodin, aloetic acid, anthranol, aloin A and B. Aloin is known to exert anti-inflammatory activity in the rat colitis, and the present extract of \textit{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. Lower levels of lipid peroxides in the brains of the drug-treated group and increased activities of enzymatic and non-enzymatic antioxidants in the brain suggest that the extract reduces oxidative stress. 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, Haloperidol are 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 and Haloperidol experimental models of Parkinson’s disease. A. vera found to be effective in increasing Rota rod performance and decreasing Catatonic response. The histologic changes induced by MPTP are also positively modulated by A. vera, so as to decrease the oxidative damage to neurons. The neuro-modulatory effect of A. vera on behavioral, oxidative stress, histologic 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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