Original Research

ALOE VERA: ITS AMERELIOATING ANTIPARKINSONIAN EFFECT IN MPTP INDUCED MOTOR AND NON-MOTOR IMPAIRMENTS IN MICE

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ABSTRACT:

Aim: *Aloe vera* (Family: Liliaceae) is known to have beneficial effects in the treatment of diabetes, skin disorders and as an anti-inflammatory agent. There is increased concern about the side effects of conventional medicine in the treatment of Parkinson's disease. Therefore, *A.vera* having antioxidative property may be a safer alternative.

Materials and Methods: The mice of either sex were divided into 06 groups (n =12). 1st group mice were given distilled water (orally), 2nd group were administered MPTP (2 doses, each dose 20 mg/kg at 2 hr. interval, i.p.).The 3rd, 4th and 5th groups were administered with *A.vera* (100, 200, and 400 mg/kg/day, orally) respectively, along with MPTP. Group 6- received Levodopa (30mg/kg, i.p,) along with MPTP. To evaluate anti-parkinsonian effect-hanging wire test, tardive dyskinesia test and elevated plus maze test were performed on the1st day and on 8th day. One way ANOVA was used to detect statistical significance followed by post-hoc Tukey test.

Results: *A.vera* (200 and 400 mg/kg, p.o.) was found to increase the hanging time significantly ($p < 0.001$) in hanging wire test and significantly decreased ($p < 0.001$) the Vacuous Chewing Movements (VCMs) in tardive dyskinesia test as compared to MPTP group. *A.vera* (200 and 400 mg/kg, p.o.) was found to significantly increase ($p < 0.001$) the time spent, no. of entries in open arm and significantly decreased ($p < 0.001$) the time spent, no. of entries in closed arm ($p < 0.001$) in elevated plus maze test when compared to MPTP group.

Conclusions: The results of the present study conclusively showed that *A.vera* has beneficial effect in MPTP induced experimental model of Parkinson's disease.

KEYWORDS: *A.vera*, MPTP, hanging wire test, tardive dyskinesia test, elevated plus maze test

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INTRODUCTION:

Till today, plants serve as an exemplary source of medicine to treat many ailments over the years[1]. *Aloe vera* (Family: Liliaceae), is one such ancient plant whose medicinal properties have been well recognised[2]. It has demonstrated better improvement in lipid profile status among rats with streptozotocin-induced diabetes[3]. In addition, recent studies revealed 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 dyskinesia's 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.

Chronic treatment with neuroleptics leads to the development of abnormal oral movements inrodents known as vacuous chewing movements (VCMs). Vacuous chewing movements in rats are widely accepted as an animal model of tardive dyskinesia[9]. The hang test can evaluate the neuromuscular strength, coordination and is sensitive to a loss of dopamine[10]. Anxiety symptoms are very common in PD patients and some author's state that anxiety in PD can be manifested even before the emergence of the first motor symptoms[11,12].
Previous studies undertaken by us shows that *A.vera* [13] possess antioxidative properties and showed beneficial effect in rotarod test and catalepsy bar tests which are behavioral models of parkinson disease. The present study was undertaken in order to further strengthen the evidence of protective role of *A. vera* in MPTP induced parkinsonism using different behavioral assessment parameters like- hanging wire test, tardive dyskinesia test, elevated plus maze test in swiss albino mice.

MATERIALS AND METHODS:

Swiss albino mice 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. The animals were housed in polypropylene cages in groups of six to eight mice per cage and kept under controlled environmental condition. All the experiments were performed at daytime between 09:30 and 15:30 hours. Care of animals was according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals. The study was duly approved by the Institutional Animal Ethics Committee, University College of Medical Sciences, and Delhi. (Approval No. IAEC/2011/49 dated 10 March 2011).

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 400 mg/kg.

The animals were divided into 06 groups (n=12).

Group I- was administered distilled water (orally, once per day ×1 weeks).

Group II- received MPTP (2 doses, each dose 20 mg/kg at 2 hr. interval, i.p. daily x 1 week).

Groups III, IV, and V- were treated with *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.

The *A.vera* (100mg/kg, 200mg/kg, 400mg/kg) orally and Levodopa (30mg/kg, i.p.) were given 30 minutes prior to MPTP administration for 08 days of experimental period. MPTP, Levodopa was obtained from Sigma Chemical Co. USA and all other chemicals used were of analytical grade.

ASSESSMENT OF BEHAVIORAL TESTS

1. Hang test[14]: Neuromuscular strength was determined in the grid hang test. Mice were lifted by their tail and slowly placed on a horizontal grid and supported until they grabbed the gird with both their fore and hind paws. The grid was then inverted so that the mice were allowed to hang upside down. The grid was mounted 20cm above a hard surface, to discourage falling but not leading to injury in case of animal fall. The apparatus was equipped with a 3-inch wall to prevent animals from transversing to the upper side of the grid. Animals were required to stay on the grid for 30 seconds. The animals were tested in the grid hang test for 30 sec and 10 chances were given with 1min interval and maximum hanging time was recorded.

2. **Tardive dyskinesia test**[15]**:** Tardive Dyskinesia is referred to as Vacuous Chewing Movements (VCMs) in rodents. On the test day mice were placed individually in a small (30× 20× 30 cm) Plexiglas cage for the assessment of oral dyskinesia. Animals were allowed 10 min to get used to the observation cage before behavioral assessments. In the present study vacuous chewing movements are referred to as single mouth openings in the vertical plane not directed toward physical material. If tongue protrusion, vacuous chewing movements occurred during a period of grooming, they were not taken into account. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for a period of 5 min.

3**. Elevated plus maze**[12]- The elevated plus-maze comprised of two open (30 cm×5 cm×0.25 cm) and two enclosed (30 cm×5 cm×15 cm) arms that radiated from a central platform (5 cm×5 cm) to form a plus sign. The maze is kept elevated 40 cm above the floor in a dimly-lit room. The test was done by placing an animal on the central platform of the maze facing an open arm. A mouse was considered to have entered an arm, when all four paws were on the arm. As a positive standard, mice were administered Lorazepam intraperitoneally (i.p). During the 5 minute test, preference of the animal for the first entry, the number of entries into the open or closed arm and the time spent in each arm of the maze were noted.

Statistical Analysis: Results of the above experiments were expressed as Mean±SD, and the difference between means was analyzed 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:

Table1. **Effect of *A.vera* on hanging wire test in MPTP treated mice.**

Groups, (Dose)	Hanging time in (Sec) -1st day	Hanging time in (Sec) -8th day
1. Distilled water(1ml/kg, p.o)	32.04 ±1.62	34.25±1.41
2. MPTP (20 mg/kg, i.p.)	10.90±1.42*	08.90±2.49*
3. *A.vera* (100mg/kg, i.p.) + MPTP	11.2 ±1.51*,‡	13.8 ±2.72*,‡
4. *A.vera* (200mg/kg, i.p.) + MPTP	15.2 ±2.41*,‡	20.5± 3.31*,†
5. *A.vera* (400mg/kg, i.p.) + MPTP	16.7±3.14*,‡	24.4±3.51*,†,‡
6.Levodopa (30 mg/kg, i.p.) +MPTP	26.2±4.61*,†	29.2±3.52*,†

The results are expressed as mean ± SD for 12 animals in each group. *$p < 0.001$ vs. distilled water- control, †$p < 0.001$ vs. MPTP, ‡$p < 0.001$ vs. (Levodopa + MPTP)

It was observed that MPTP alone treated group, significantly decreased the hanging time ($p<0.001$) on 1st day and on 8th day as compared to control group. In Levodopa treated group, significant increase in hanging time ($p<0.001$) was seen on both 1st day and on 8th day, as compared to MPTP treated group. *A.vera* 100mg/kg, 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in hanging time on 1st day. But on 8th day, *A.vera* 200 mg/kg and 400mg/kg pretreated groups showed significant increase in hanging time ($p<0.001$) when compared to MPTP (as shown in table 1).Whereas no significant difference in hanging time was seen when *A.vera* 400 mg/kg treated group compared to levodopa treated-group.

Table 2:**Effect of *A.vera* on tardive dyskinesia in MPTP treated mice.**

Groups, (Dose)	VCMs/5 min -1st day	VCMs/5 min -8th day
1. Distilled water (1ml/kg, p.o)	8.2±1.51	9.4±1.21
2. MPTP (20 mg/kg, i.p.)	50.7+3.91*	55.6+3.63*
3. *A.vera* (100mg/kg,i.p.) +MPTP	48.2+1.23*,‡	40.8+2.59*,‡
4. *A.vera* (200mg/kg,i.p.) +MPTP	47.5±3.28*,‡	20.2±4.37*,†,‡
5. *A.vera* (400mg/kg,i.p.) +MPTP	44.7±3.18*,‡	17.3±2.25*,†
6.Levodopa (30mg/kg,i.p.) + MPTP	14.3±4.37*,†	12.5±4.05*,†

The results are expressed as mean ± SD for 12 animals in each group. *p < 0.001 vs.distilled water - control, †p < 0.001 vs. MPTP, ‡p < 0.001 vs. (Levodopa + MPTP).

It was observed that among MPTP alone treated group, significant increase p<0.001 in vacuous chewing movements (VCMs) was seen on 1st day and on 8th day when compared to control group. In Levodopa treated group, significant decrease in (VCMs) p<0.001 was seen on 1st day and on 8th daywhen compared to MPTP treated group. *A.vera* 100mg/kg, 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in (VCMs) on the 1st day. But on 8th day, *A.vera* 200mg/kg and 400mg/kg pretreated groups showed significant decrease in (VCMs) p<0.001 when compared to MPTP treated group (as shown in table 2), whereas no significant difference in (VCMs) was seen when *A.vera* 400 mg/kg treated group compared to levodopa treated group.

Table 3:Effect of single dose observation in Elevated Plus maze test on 1st day

Groups, (dose)	No. of entries in O.A	Time spent in O.A (S)	No. of entries in C.A	Time spent In C.A (S)
1. Distilled water (1ml/kg, p.o)	4.2 ±0.31	25.5 ±1.57	18.21 ±1.12	267.2 ±3.42
2. MPTP (20 mg/kg, i.p.)	1.4 ±0.45*	9.26 ±2.11*	3.5 ± 2.64*	260.4± 0.75*
4. A.vera (200mg/kg,i.p.) +MPTP	5.3±1.02*, ‡	33.2 ±2.58*, ‡	16.9±0.67*, ‡	255.7±2.92*, ‡
5. A.vera (400mg/kg,i.p.) +MPTP	15.2±0.73*, ‡	203.7 ±5.82*, ‡	6.5 ±0.51*, ‡	98.5 ±2.27*, ‡
6.Levodopa(30mg/kg,i.p.)+MPTP	17.6±1.17*, ‡	247.5± 2.58*, ‡	5.7±0.36*, ‡	53.3±2.12*, ‡

O.A-Open arm, C.A-Closed arm, S-seconds, (n=6).The results are expressed as mean ± SD.*p < 0.001 vs. distilled water - control, †p < 0.001 vs. MPTP, ‡p < 0.001 vs. (Levodopa + MPTP)

Table 4:Effect of single dose observation in Elevated Plus maze test on 8th day

Groups, (dose)	No. of entries in O.A	Time spent in O.A (S)	No. of entries in C.A	Time spent In C.A (S)
1. Distilled water (1ml/kg, p.o)	6.4±0.38	24.5±0.85	16.3 ±1.42	272.3 ±14.46
2. MPTP (20 mg/kg, i.p.)	1.7 ± 0.49*	10.6 ± 2.18*	4.7 ± 2.68*	261.5±0.74*
4. *A.vera* (200mg/kg,i.p.) +MPTP	13.5±1.16*,†	197.2±4.02*,†	10.5±0.62*,†	126.6±1.95*,†
5. *A.vera* (400mg/kg,i.p.) +MPTP	18.2± 1.23*	213.7 ±6.93*	6.8 ± 0.43*	85.5 ± 1.82*
6.Levodopa(30mg/kg,i.p.)+MPTP	20.2 ± 0.81‡	263.5± 5.04‡	5.4 ±0.41‡	40.6 ±1.12‡

O.A-Open arm, C.A-Closed arm, S-seconds, (n=6). The results are expressed as mean ± SD.*p < 0.001 vs. distilled water - control, †p < 0.001 vs. MPTP, ‡p < 0.001 vs. (Levodopa + MPTP)

In group treated with MPTP, there was significant decrease (p<0.001) in number of entries & time spent in open arm and significant increase (p<0.001) in number of entries & time spent in closed arm (p<0.001) on 1st day and on 8th day as compared to control group (as shown in table 3 and table 4). The mice which received *A.vera* (200, 400mg/kg,i.p.) did not show significant antianxiety effect when compared to control and MPTP treated groupson 1st day. Whereas, on 8thday there was significant increase (p<0.001) in number of entries & time spent in open arm and significant decrease (p<0.001) in number of entries & time spent in closed arm (as shown in table 3 and table 4).The mice which received *A.vera* (400mg/kg,i.p.) showed antianxiety effect, there was no significant difference when compared to levodopa treated group (as shown in table 3 and table 4). On1st day and on 8th day,Levodopa treated group , animals showed significant increase (p<0.001) in number of entries, time spent in open arm and significant decrease (p<0.001) in number of entries,time spent in closed arm when compared to control and MPTP treated groups (as shown in table 3 and table 4).

DISCUSSION:

Parkinson's disease is a chronic neuro - degenerative characterized by resting tremor, bradykinesia, shuffling gait, flexed posture and rigidity. While the cause of the degeneration is not known, oxidative stress plays a vital role[8]. Oxidative stress may arise from the metabolism of dopamine with the production of potentially harmful free radical species[17]. Compared to the rest of brain, the substantia nigra pars compacta is exposed to a higher rate of reactive oxygen species 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[18]. Various studies have reported oxidative stress changes in the brain of Parkinson's disease patients[19].

(MPTP), 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine is a potent neurotoxin used to create an experimental model of Parkinson's disease in animals. Certain aspects of the Parkinson's disease such as catalepsy, motor incoordination and bradykinesia can be easily studied in this model. As MPTP is highly lipophilic, makes it enable to cross the blood brain barrier immediately after its systemic absorption. Once MPTP reaches the brain tissue, it is converted to the hydrophilic metabolite 1-methyl-4 phenylpyridinium ion (MPP^+), the free radical reactive specie in the causation of dopaminergic neuronal loss. It is established that these free radical reactive species play a vital role in the pathogenesis of dopaminergic neuronal loss in Parkinson's disease[20].

Although the mechanisms of cell death induced by MPP+ have not been fully characterized, it is known that MPP+ is an effective inhibitor of complex I respiration in isolated mitochondria. As a result, a rapid decrease in adenosine triphosphate (ATP) content occurs in the striatum and substantia nigra pars compacta (SNpc), the brain regions most sensitive to MPTP-induced neurotoxicity[21].

In the present study, three behavioral assessment parameters – hanging wire test, tardive dyskinesia, elevated plus maze tests were used to assess MPTP induced Parkinson disease amice. The mice when pretreated with *A.vera* (200, 400 mg/kg, p.o.) for 08 days, significantly increased the hanging time in hanging wire test, decreased the vacuous chewing movements (VCMs) in tardive dyskinesia test and this effect is comparable to that of levodopa group. The above findings of behavioral tests are similar with other previous studies[22,23].

The mice when pretreated with *A.vera* (200, 400 mg/kg, p.o.) for 08 days, significantly increased ($p<0.001$) the number of entries & time spent in open arm and significant decrease ($p<0.001$) in number of entries & time spent in closed arm and this effect is comparable to that of levodopa group. The above findings of behavioral tests are similar with other previous studies[24-26].

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[27]. 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[28]. Recently, involvement of inflammatory process has been also reported in the pathogenesis of Parkinson's disease[18,28]. 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[29].

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 property [4,5,30]. *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 has shown beneficial effects in our present study behavioral assessment parameters of experimental models of parkinson disease. 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 in haloperidol and MPTP induced parkinsonian animal models[31,32]. Thus further studies are required to clearly establish its role as an anti-parkinson agent.

CONCLUSION:

MPTP is a potent neurotoxin is commonly used to create experimental model of Parkinson's disease. The results of the present study conclusively showed that *A.vera* has beneficial effects in hang wire test, tardive dyskinesia test and elevated plus maze test. 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.

REFERENCES:

1. Ates DA, Erdogrul OT., Antimicrobial activities of various medicinal and commercial plant extracts., Turk J Biol.,2003;27:157-162.
2. Shelton RM., Aloe vera: its chemical and therapeutic properties., Int J Dermatol.,1991;30:679-683.
3. Rajasekaran S, Ravi K, Sivagnanam K, Subramanian S., Beneficial effects of *Aloe vera* leaf gel extract on lipid profile status in rats with streptozotocin diabetes., Clin Exp Pharmacol Physiol.,2006;33:232-237.
4. Halder S, Mehta AK, Mediratta PK., Augmented humoral immune response and decreased cell-mediated immunity by Aloe vera in rats., Inflammopharmacology.,2012;20:343–346.
5. Rathor N, Mehta AK, Sharma AK, Mediratta PK, Sharma KK., Acute effect of Aloe vera gel extract on experimental models of pain., Inflammation.,2012;35:1900–1903.
6. Parihar MS, Chaudhary M, Shetty R, Hemnani T., Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice: prevention by extracts of Withania somnifera and Aloe vera., J Clin Neurosci.,2004;11:397–402.
7. Agid Y, Ruberg M, Javoy-Agid F, Hirsch E, Raisman-Vozari R, Vyas S, et al., Are dopaminergic neurons selectively vulnerable to Parkinson's disease?., Adv Neurol.,1993;60:148-164.
8. Prasad KN, Cole WC, Kumar B., Multiple antioxidants in the prevention and treatment of Parkinson's disease., J Am Coll Nutr.,1999;18:413-423.
9. Naidu PS, Kulkarni SK., Excitatory mechanisms in neuroleptic-induced vacuous chewing movements (VCMs): possible involvement of calcium and nitric oxide.,Behav Pharmacol., 2001;12(3):209-16.
10. Tillerson JL, Miller GW., Grid performance test to measure behavioral impairment in the MPTP-treated mouse model of parkinsonism., J Neurosci Meth., 2003;123:189-200.
11. Dissanayaka NNW, Sellbach A, Matheson S, O'Sullivan JD, Silburn PA, Byrne GJ, Marsh R, Mellick GD., Anxiety Disorders in Parkinson's Disease: Prevalence and Risk Factors., Mov Disord., 2010;25:838–845.
12. Chaudhuri KR, Healy DG, Schapira AH., Non-motor symptoms of Parkinson's disease: diagnosis and management., Lancet Neurol., 2006;5:235–245
13. Harish G Bagewadi, Naveen Rathor., Effect of *Aloe vera* on Animal models of Parkinson disease in Mice., Int J Pharm Bio Sci., 2014;5(3):549 –559.
14. Tillerson JL, Miller GW., Grid performance test to measure behavioural impairment in the MPTP treated mouse model of Parkinsonism.,J NeurosciMeth.,2003; 123:189-200.
15. Rogoza RM, Fairfax DF, Henry P, N-Marandi S, Khan RF, Gupta SK, Mishra RK., Electron spin resonance spectroscopy reveals alpha-phenyl-N-tert-butylnitrone spin-traps free radicals in rat striatum and prevents haloperidol-induced vacuous chewing movements in the rat model of human-tardive dyskinesia., Synapse., 2004 ;54:156–163.
16. Wei XY, Yang JY, Wang JH, Wu CF., Anxiolytic effect of saponins from Panex quinquefolium in mice., Journal of Ethno pharmacology., 2007;111:613-618.
17. Yuan H, Zheng JC, Liu P, Zhang SF, Xu JY, Bai LM., Pathogenesis of Parkinson's disease: oxidative stress, environmental impact factors and inflammatory processes., Neurosci Bull., 2007;23:125–130.
18. Slivka A, Cohen G., Hydroxyl radical attack on dopamine., J Biol Chem.,1985;260:15466-15472.
19. Sriram K, Pai KS, Boyd MR, Ravindranath V., Evidence for generation of oxidative stress in brain by MPTP: in vitro and in vivo studies in mice., Brain Res.,1997;749:44-52.

20. Yoshikawa T., Free radicals and their scavengers in Parkinson's disease., Eur. Neurol., 1993;33:60–68.
21. Chan P, DeLanney LE, Irwin I, Langston JW, Di Monti D., Rapid ATP loss caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mouse brain., J Neurochem., 1991;57:348–351.
22. Mohanasundari M., Sethupathy.S. , Sabesan.M., The effect of Hypericum perforatum extract against the neurochemical and behavioral changes induced by 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice., Indian J Pharmacol., 2006; 38 (4): 266-270.
23. Monalisa Jenaa, Swati Mishra, Abhisek Palb, Sudhanshu Sekhar Mishra., Protective effect of *Eclipta alba* on haloperidol induced extrapyramidal movement disorders in albino rats.,J. Chem. Pharm. Res., 2014; 6(7):31-38.
24. George S, van den Buuse M, San Mok S, Masters CL, Li QX, Culvenor JG., Alpha-synuclein transgenic mice exhibit reduced anxiety like behaviour., Exp-Neurol.,2008 ; 210: 788–92.
25. Taylor TN, Caudle WM, Shepherd KR, Noorian A, Jackson CR, Iuvone PM, et al., Nonmotor symptoms of Parkinson's disease revealed in an animal model with reduced monoamine storage capacity., J Neurosci.,2009;29:8103–13.
26. Filipa L. Campos et al., Rodent models of Parkinson's disease: beyond the motor symptomatology., Front Behav Neurosci., 2013; 7: 175.1-11.
27. Zhang XF, Wang HM, Song YL, Nie LH, Wang LF, Liu B, et al., Isolation, structure, elucidation, antioxidative and immunomodulatory properties of two novel dihydrocoumarins from Aloe vera., Bioorg Med Chem Lett., 2006;16:949-953.
28. Aubin N, Curet O, Deffois A, Carter C., Aspirin and salicylate protect against MPTP-induced dopamine depletion in mice., J Neurochem.,1998;71:1635-1642.
29. Teismann P, Vila M, Choi DK, Tieu K, Wu DC, Jacksonlewis V, Przedborski S., COX-2 and Neurodegeneration in Parkinson's Disease., Ann N Y Acad Sci.,2003;991:272–277.
30. Vazquez B, Avila G, Segura D, Escalante B., Antiinflammatory activity of extracts from Aloe vera gel., J Ethnopharmacol.,1996;55:69–75.
31. M.Vishnu Vardhan Reddy, Manmohan Singhal., Evaluation of Anti-Parkinsonian activity of seed extract of Nelumbo Nucifera., Int J Pharm Bio Sci., 2014;5(2):469 – 485.
32. Harish G. Bagewadi, Afzal Khan AK., Evaluation of anti-parkinsonian activity of *Elaeocarpus ganitrus* on haloperidol induced Parkinson's disease in mice., Int J Basic Clin Pharmacol., 2015;4:102-6.

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