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Evaluation of Anti-Parkinson Activity of Aqueous Extract of *Barleria prionitis*

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Abstract

AIM- The aim of the preset investigation is Evaluation of Anti-Parkinson Activity of Aqueous Extract of *Barleria prionitis*. **MATERIAL & METHODS-** The whole plant of *Barleria prionitis*were collected from the surrounding areas of Erode district, Tamilnadu, India during the month of December. Coarsely powdered whole plant of *Barleria prionitis* were extracted with water for 48 hours at room temperature. After extraction the extracts were evaporated by using rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4 ^{o}C for the further analysis. Male Swiss albino mice 3 month of age, and 25-30 g body weight were offered. All the rats were kept at room temperature and allowed to acclimate in standard conditions less than 12 hr light/ 12 hr dark cycle in the animal house. The MPTP was purchased from sigma chemicals, Mumbai, India and was stored according to the manufacturer label (37^{0}C) to prevent its decomposition. The length of time (duration) the animal stay on the rod without falling, gives a measure of their coordination, balance, physical condition and motor-planning. RESULTS- The grip strength of treated rats were found to be increased in the rats as compared to untreated rats. Similarly, Locomotor activity were also significantly increased in the rats treated in rats as compared to untreated rats. **DISCUSSION-** From the results the Actophotometer readings (locomotor activity) of animals of vehicle-treated control group (Group I) was found to be 386.33±1.56 and 396±1.15 counts/5 min for all 7 days of treatment. MPTP treatment to animals of Group II showed a significant reduction in locomotor activity every week. The actophotometer readings decreased to 186±2.30 counts/ 5 min. on the 7th day, went down to 145.33±0.23 counts/5 min. Thus, there was a significant decline in the locomotor activity of rotenone treated control animals (Group III) when compared to vehicle-treated control group (Group I). **CONCLUSION**- From the present study, it can be considered that the aqueous extract of *Barleria prionitis* exhibited significant anti-parkinsonism activity in MPTP model in mouse and rats respectively.

KEYWORDS- Anti-Parkinson Activity, Aqueous Extract, *Barleria prionitis,* Parkinson Disease, Muscular rigidity, Bradykinesia

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INTRODUCTION

Parkinson Disease (PD) is the second most chronic neurodegenerative disorder in the world, after Alzheimer´s Disease (AD), and is estimated to affect about 2% of the population over 60 years of age. The disease generally affects persons aged 55–64 years, although occasionally much younger individuals are affected. Although the causes for degeneration of dopaminergic neurons in Parkinson's disease are not well understood, The degeneration of these dopaminergic neurons leads to four cardinal, debilitating symptoms: resting tremor, muscular rigidity, bradykinesia, and postural imbalance.[1,2]

At current research, the etiology of PD is still not clearly known. Evidences suggest massive oxidative stress leading to the formation of free radical. Pathologically the hallmark of Parkinson disease are the severe loss of dopaminergic neuron in the substantia nigra pars compacta and the presence of proteinaceous inclusion called lewy bodies, which is mainly composed of fibrillar α-synuclein and ubiquitinated protein with in some remaining nigral neurons.[3,4]

Various literature and studies show that the active principles triterpenoids, iridoids , and flavonoids are having a crucial role in anti-parkinson treatment. *Barleria prionitis* is rich in these types of triterpenoids, Iridoids, and Flavonoids. The purpose of this study is to investigate and evaluate anti-parkinson activity on MPTP and Rotenone-induced model by using aqueous extracts of *Barleria prionitis.*

MATERIAL AND METHODS

COLLECTION AND AUTHENTICATION OF PLANT

The whole plant of *Barleria prionitis*were collected from the surrounding areas of Erode district, Tamilnadu, India during the month of Decemberand authenticated by Botanical survey of India (BSI) southern circle, Coimbatore, Tamilnadu. Soon after collection the aerial parts were cleaned, driened in shade and crushed to a coarse powder, stored in an

air tight plastic container, until further use.

EXTRACTION OF PLANT MATERIAL

Coarsely powdered whole plant of *Barleria prionitis* were extracted with water for 48 hours at room temperature. After extraction the extracts were evaporated by using rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4 ^{o}C for the further analysis.

Evaluation of Anti-Parkinson Study of Aqueous Extract of *Barleria prionitis linn*

Male Swiss albino mice 3 month of age, and 25-30 g body weight were offered. All the rats were kept at room temperature and allowed to acclimate in standard conditions less than 12 hr light/ 12 hr dark cycle in the animal house. Animals are fed with commercial pellet diet and water ad libitum freely throughout the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee).

EXPERIMENTAL DESIGN FOR MPTP INDUCED PARKINSON

Table 1: Experimental design for MPTP induced Parkinson

Group	Number of animals	Group Specifications
Group I	6	Vehicle control (normal saline
Group II	6	Only MPTP (25 mg/kg, i.p)
Group III	6	MPTP + Standard [Levodopa
Group IV	6	MPTP + AEBP (200 mg/kg,
Group V	6	MPTP + AEBP (400 mg/kg,

Induction of Parkinson[5,6]

Preparation and induction of MPTP solution

The MPTP was purchased from sigma chemicals, Mumbai, India and was stored according to the manufacturer label (37^{0}C) to prevent its decomposition. The MPTP solution was freshly prepared at 25 mg/kg. The MPTP was dissolved in 0.9% sodium chloride solution and injected i.p at the dose of 25 mg/kg body weight, 7 days. MPTP solution is stable only for a period of 24 hours at 4^{0}C.

Preparation of Levodopa and Benzerazide

12mg/kg of levodopa and 3 mg/kg of Benzerazide was dissolved in distilled water. Levodopa and benzerazide was freshly prepared daily and given via i.p to the standard group.

Preparation of sample

200 mg/kg and 400 mg/kg were dissolved in distilled water and it was prepared freshly and given via oral route to group IV& V respectively for 7 days.

EVALUATION PARAMETERS

MOTOR CO-ORDINATION TEST (ROTA ROD TEST)

The length of time (duration) the animal stay on the rod without falling, gives a measure of their coordination, balance, physical condition and motor-planning.[7]

Motor Co-ordination test was conducted using rota rod apparatus. Animal was placed individually on the rotating rod and trained for 3 min trail at 25 rpm on the day before the first day of testing. A cut off time of 180s was fixed and each animal performed 3 separate trials at 5 min interval. After each trial, 5 min rest period was given to alleviate stress and fatigue. Motor coordination can be tested by comparing the latency to fall on the very first trial between treatment groups. The time taken by animals to fall from the rotating rod was noted.[8]

LOCOMOTOR ACTIVITY

An actophotometer could have either circular or square area in which the animal moves.[9]

The spontaneous locomotor activity of each animal was recorded individually, using Actophotometer. The apparatus was placed in a sound attenuated and ventilated room during the testing period. All the animals were placed individually in the activity cage for 3 min to

habituate them before starting actual locomotor activity task for the next 3 min. the basal activity score was noted. The units of the activity counts were arbitrary and based on the beam breaks by movement of the animal. Counts/3 min is used as an index of locomotor activity.

FORCED SWIMMING TEST

The time spent by the animal as immobile in water represents the depression-like behaviour.[10]

The test was performed according to the method described by Porsolt et al., 1977, with slight modifications. Animals were forced to swim in a glass cylinder (20 cm height, 14 cm diameter) containing 10 cm depth of water at 25° c. After the initial 2 min acclimatization period, the total duration of immobility was measured during final 4 min of the 6 min test session. Animal were considered to be immobile, when they made no further attempts to escape except the movements necessary to kept their heads above the water. After 6 min, the animals were removed from water, allowed to dry, and returned back to their home cage.[11]

HOLE BOARD TEST

Decrease in anxiety shows increased exploration of the holes. Whereas increased anxiety shows lower number of head poking.[12]

The hole board apparatus consist of a wooden board (40*40cm) placed 25 cm above the ground. It consists of 16 holes which is about 3 cm in diameter, spaced symmetrically in a diamond pattern. Animals were placed on the corner of the apparatus and were observed for the next 5 min for the number of head dipping. A head dipping is counted when the animal introduces its head into any hole of the box up to the level of the ears. The apparatus was

thoroughly cleaned between each subject.[13]

TAIL SUSPENSION TEST

The test is based on the principle that animal subjected to the short -term, inescapable stress of being suspended by their tail, will develop an immobile posture.[13]

The tail suspension test is another well characterized test for assessing depression-like and anti-depressant like activity. In this test animal were individually suspended by the tail to a horizontal ring –stand bar (distance from floor = 30cm) using adhesive tape (distance from tip of tail = 2cm). Typically animal demonstrated several escape-orientated behaviours interspersed with temporally increasing bouts of immobility. A 6-mins test session was employed, which was videotaped.

The parameter recorded was the number of seconds spent immobile.[14]

***In vivo* antioxidant activity**

Estimation of reduced glutathione (GSH)

To 1 ml of the homogenate, 1 ml of the TCA solution was added and centrifuged. The supernatant was collected and the precipitate formed was removed. To 0.5 ml of supernatant 2 ml of DTNB was added, the volume was made up to 3 ml with phosphate buffer. Then absorbance was read at 412 nm. The amount of glutathione was expressed as µ/mg protein.[15]

Determination of lipid peroxidation

To 0.1 ml of sample, 2 ml of TBA-TCA-HCl reagent (ratio of 1:1) was added mixed and kept in a water bath for 15 minutes. Afterward the solution was cooled and supernatant was removed and absorbance was measured at 535 nm against reference blank. The level of lipid peroxides was given as nm moles of MDA formed/mg protein.[16, 17]

STATISTICAL ANALYSIS

The statistical analysis was carried out by using PRISM version 5 software. The data's of all parameters were analysed by means of one way ANOVA followed by Dunnett's test. The results were expressed as mean ±SEM.

RESULTS

SCREENING OF ANTIPARKINSONIAN ACTIVITY OF AEBP:

ROTA ROD TEST:-

Table 1: Effect of AEBP on muscle grip strength

Groups	Time spent on Rota rod (sec)		
	Day 3	Day 5	Day 7
Vehicle control	178±0.57	179±1.48	178.67±0.95
MPTP	74.667±1.33***	62±0.26***	61.333±0.18***
MPTP+ Levodopa/benzerazide	59±2.02***	84.667±1.76***	120.67±0.59***
MPTP + AEBP (200 mg/kg)	47±1.52***	67±0.64***	94.667±0.72***
MPTP + AEBP (400 mg/kg)	53.333±2.40***	81±2.08***	111.67±0.14***

Statistical comparison: Each group (n=6), * P<0.05, ** P<0.01, and *** P<0.001 denotes comparison of all groups with parkinsonic control.

ACTOPHOTOMETER TEST:

Table 2:- Effect of AEBP on Spontaneous locomotor activity

Groups	Locomotive score (sec)		
	Day 3	Day 5	Day 7
Vehicle control	384±3.60	394±1.15	386.33±1.56
MPTP	186±2.30***	162.67±2.02***	145.33±0.23***
MPTP+ Levodopa/benzerazide	166.33±2.60***	217.33±0.02***	246.33±0.77***
MPTP + AEBP (200 mg/kg)	155.33±1.48***	184.67±0.91***	215.67±0.56***
MPTP +AEBP (400 mg/kg)	162±1.55***	198±1.12***	236.67±1.85***

Statistical comparison: Each group (n=6), * P<0.05, ** P<0.01, and *** P<0.001 denotes comparison of all groups with parkinsonic control.

FORCED SWIM TEST:

Table 3: Effect of AEBP on depression

Groups	Total immobility time (sec)		
	Day 3	Day 5	Day 7
Vehicle control	52.333±2.60	47.667±0.93	48.667±1.33
MPTP	125±0.73***	130.33±1.76***	131.33±2.37***
MPTP+ Levodopa/benzerazide	117.67±0.06***	101±1.15***	85.333±1.78***
MPTP + AEBP (200 mg/kg)	127.67±2.85***	120.67±0.89***	118.67±2.27***
MPTP +AEBP (400 mg/kg)	127±1.01***	116.33±3.74***	93.333±0.15***

Statistical comparison: Each group (n=6), * P<0.05, ** P<0.01, and *** P<0.001 denotes comparison of all groups with parkinsonic control.

TAIL SUSPENSION TEST:-

Table 4: Effect of AEBP on depression

Groups	Immobility time (sec)		
	Day 3	Day 5	Day 7
Vehicle control	54.667±1.29	52.667±1.85	53±1.52
MPTP	129.67±3.33***	132.67±0.04***	131±2.47***
MPTP+ Levodopa/benzerazide	123±2.08***	103.67±1.66***	87.667±0.18***
MPTP + AEBP (200 mg/kg)	132.67±0.36***	123.33±1.45***	121.33±4.93***
	128.33±2.12***	118.33±1.48***	96±0.83***

Statistical comparison: Each group (n=6), * P<0.05, ** P<0.01, and *** P<0.001 denotes comparison of all groups with parkinsonic control.

HOLE BOARD TEST:-

Table 5: Effect of AEBP on alertness

Groups	No. of head dippings		
	Day 3	Day 5	Day 7
Vehicle control	36±1.31	36±0.14	33.667±2.46
MPTP	22±3.68***	22.333±1.79**	18.333±4.82***
MPTP+ Levodopa/benzerazide	23±1.67***	24±2.09**	31.333±0.88ns
MPTP + AEBP (200 mg/kg)	18.333±3.36***	18±1.73***	21.333±0.91***
MPTP +AEBP (400 mg/kg)	23.333±4.55***	24.333±2.52**	27.333±1.20*

Statistical comparison: Each group (n=6), * P<0.05, ** P<0.01, and *** P<0.001 denotes comparison of all groups with parkinsonic control.

***IN VIVO* ANTIOXIDANT STUDY**

Table 6: Effect of AEBP on *IN VIVO* antioxidants of brain of MPTP induced parkinsonic mice.

Groups	thione µg/mg protein)	l of MDA/mg protein)
Vehicle control	6.4933±0.0014	6.1547±0.0588
	2.34±0.0556***	11.436±0.0067***
MPTP+ Levodopa/benzerazide	4.225±0.0565***	7.2437±0.0014***
MPTP + AEBP (200 mg/kg)	3.2367±0.0638***	9.055±0.0062***
MPTP +AEBP (400 mg/kg)	4.1457±0.0560***	7.5247±0.0075***

Statistical comparison: Each group (n=6), * P<0.05, ** P<0.01, and *** P<0.001 denotes comparison of all groups with parkinsonic control.

DISCUSSION

This study aims to investigate the Neuroprotective effect of *Barleria prionitis* on MPTP intoxicated animal models of PD by analyzing behavior patterns, brain antioxidant studies. *Barleria prionitis* has a long history in herbal medicine in various countries. The leaves and bark of *Barleria prionitis* have long been used as a natural medicine in tropics. The bark and leaf extract of *Barleria prionitis* is well known for its different types of pharmacological properties such as Stomach disorder, Urinary infections, fever, Tooth ache, Diuretic, Jaundice, Asthma, Arthritis, Inflammation, Migraine, Dropsy, Leprosy.

Exposure to the pesticide rotenone is linked to an increased risk of PD in epidemiological studies[18], and have been adapted for PD models. Actophotometer is used for screening the locomotor and anti-anxiety activity in rodents, while the rotarod for muscle relaxant activity. Locomotor activity indicates attentiveness and the decline indicates sedative action. The GABA receptor compound is concerned in sedation, muscle relaxant and anxiety in CNS. Various neurological and mental disorders such as epilepsy, depression, Parkinson syndrome, Alzheimer's disease are involved with this receptor. The increase in locomotor activity OF extract of *Barleria prioitis* has shown stimulant effect in actophotometer. This provoked to evaluate it further, using paradigms of depression models. From the results the Actophotometer readings (locomotor activity) of animals of vehicle-treated control group (Group I) was found to be 386.33±1.56 and 396±1.15 counts/5 min for all 7 days of treatment. MPTP treatment to animals of Group II showed a significant reduction in locomotor activity every week. The actophotometer readings decreased to 186±2.30 counts/ 5 min. on the 7th day, went down to 145.33±0.23 counts/5 min. Thus, there was a significant decline in the locomotor activity of rotenone treated control animals (Group III) when compared to vehicle-treated control group (Group I). Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced significant increase in locomotion (246.33±0.77, 215.67±0.56, 236.67±1.85s counts/ 5 min.) on the 7th day of treatment; as compared to the MPTP treated control (Group II) animals on the respective days. Rota rod test a standard animal model used to evaluate peripheral neuromuscular blockade and the motor coordination, a deficit in motor coordination would very likely affect performance in the behavioral tests. Rota rod test, the difference in the fall of time from the rotating rod between the vehicle and extract treated groups were taken as an index of muscle relaxation. From the results the Rota rod readings (fall off time) of Vehicle-treated Control group (Group I) animals from the rota rod was found to be

178.67±0.96. MPTP treatment to animals of Group II showed a significant reduction in the muscle grip or strength every week. The rotarod fall off times decreased to 74.667±1.33s. counts/5 min. on the 7th day, went down to 61.333±0.18 counts/5min. Thus, there was a significant decrease ($p<0.001$) in the muscle activity of MPTP treated control animals (Group II) when compared to vehicle treated control group (Group I). Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced significantly increased the muscle activity (120.67±0.59, 94.667±0.72, 111.67±0.14s) on the 7th day of treatment; as compared to the MPTP treated control (Group II) animals on the respective days. Due to their immobility time, muscular coordination skill memory, motor impairment, retention times were also decreased. From the results the Tail suspension test readings (total immobility time) of Vehicle-treated Control group (Group I) animals was found to be 53±1.52. MPTP treatment to animals of Group II showed a significant increase in the immobility time every week. The tail suspension immobility times increased to 129.67±3.33. sec/6 min. on the 7th day, went up to 131±2.47 sec/6min. Thus, there was a significant increase ($p<0.001$) in the immobility time of MPTP and Rotenone treated control animals (Group II) when compared to vehicle treated control group (Group I). Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced significantly decreased the muscle activity (87.667±0.18, 121.33±4.93, 96±10.83s) on the 7th day of treatment; as compared to the MPTP treated control (Group II) animals on the respective days.

Forced swimming test readings (total immobility time) of Vehicle-treated Control group (Group I) animals was found to be 48.667±1.33 and 53±1.94s. MPTP and Rotenone treatment to animals of Group II showed a significant increase in the immobility time every week. The immobility times increased to 125±0.73 and 128.67±0.85 s. sec/6 min. on the 7th day, went up to 131.33±2.37 and 132.67±1.45s sec/6min. Thus, there was a significant increase ($p<0.001$) in the immobility time of MPTP and Rotenone treated control animals (Group II) when compared to vehicle treated control group (Group I). Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced significantly decreased the muscle activity (85.333±1.78, 118.67±2.27, 93.333±0.15s) on the 7th day of treatment; as compared to the MPTP treated control (Group II) animals on the respective days. From the results the Hole board test readings (No.of head dippings) of Vehicle-treated Control group (Group I) animals was found to be 33.667±2.46. MPTP treatment to animals of Group II showed a significant decrease in the head dippings every week. The hole board test head dipping decreased to 22±3.68 sec/5 min. on the 7th day, went up to 18.333±4.82s sec/5min. Thus, there was a significant decrease ($p<0.001$) in the head dipping of MPTP and Rotenone treated control animals (Group II) when compared to vehicle treated control group (Group I).Treatment and Pretreatment with standard drug, *Barleria prionitis* (Group III,IV,V) produced significantly increased the head dipping (31.333±0.88, 21.333±0.91, 27.333±1.20) on the 7th day of treatment; as compared to the MPTP treated control (Group II) animals on the respective days. The biotransformation of MPTP into MPP+, which is catalyzed by the mitochondrial enzyme monoamine oxidase B, represents the major route for MPTP-mediated neurotoxicity. The conversion of MPTP to MPP+ has been suggested to induce the formation of ROS. This notion is supported by previous studies which showed increased superoxide ($O2\cdot-$) and hydroxyl radical ($\cdot OH$) levels during the biotransformation of MPTP. While the damage induced by $O2\cdot-$ is limited, it can react with nitric oxide (NO) to form peroxynitrite ($ONOOO-$) which readily forms the more reactive $\cdot OH$ radical. Other studies have shown that MPTP induces toxicity through ATP depletion and mitochondrial dysfunction. Moreover, that ATP depletion plays a major role in MPTP induced neuronal cell death [19]. However, it is likely that MDA can form complexes with other biological components such as protein, lipids, and nucleic acids which can contribute to an underestimation of endogenous lipid peroxidation [20]. On the contrary to our lipid peroxidation data, we also show that MPTP can lead to distinct alterations in endogenous antioxidant defense mechanisms. MPTP treatment has been previously shown to significantly increase Mn-SOD and CuZn-SOD activities in the striatum of C57BL/6 mice, which is suggestive of acute oxidative stress insult.

MPTP selectively damages the dopaminergic nigrostriatal system, resulting in the loss of dopaminergic neurons in the substantia nigra and a depletion of dopamine in the striatum. The loss of dopaminergic neurons is associated with an onset of motor symptoms, and there is a direct relationship between extent of dopamine loss and motor dysfunction. The striatum is considered to be the region responsible for head and forelimb motor control. Many studies have revealed impaired behavioural responses within a short span of MPTP lessoned animals. The neurotransmitter, DA plays a key role in body movement and motor control. A well known fact is that there is a reduced level of Dopamine occurring in dopaminergic neuronal damaged brain.

CONCLUSION

From the present study, it can be considered that the aqueous extract of *Barleria prionitis* exhibited significant anti-parkinsonism activity in MPTP model in mouse and rats respectively. The probable mode of action of this plant decreased lipid peroxidation due to the presence of flavonoids, polyphenols and glycosides. All the Parameters of extract treated group animals have shown better results when compared with MPTP induced group and the standard L-dopa treated group.

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