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Assesment of Anti-Parkinsonian Activity of Different Extracts of *Barleria prionities* Root

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ABSTRACT

The aim of the present study was to evaluate neuroprotective effect of different extracts of *Barleria prionities* (BP) roots (Acanthaceae) in MPTP (1-methyl,4-phenyl-1,2,3,6-tetrahydropyridine) mouse model. BP root extracts was administered at different doses of 100, 200 and 300 mg/kg (P.O) in different groups once a day for seven days and the first dose was given 30 min prior to first MPTP injection. Behavioural parameters were assessed on 1^{st}, 3^{rd} and 6^{th} day of treatment. The alcoholic extract at given doses significantly dose dependently increased the spontaneous motor activity, grip strength and alertness. Mice were sacrificed by decapitation and whole brain was analysed for dopamine, norepinephrine, epinephrine, serotonin, reduced glutathione level and lipid peroxidation. Alcoholic, aqueous and ethyl acetate extract of BP significantly ($P<0.001$) improved the brain dopamine and other monoamines like norepinephrine, epinephrine and serotonin and also improved the reduced glutathione level and decreased the lipid peroxidation level as compared to MPTP control group in a dose dependent manner, while petroleum ether fraction had moderate effect. The results of the present study indicated that the alcoholic and aqueous extract of BP root possess significant neuroprotection which may be due to presence of L-dopa, flavonoids and phenolic compounds which might cross the blood brain barrier.

Key words: Parkinsonism, *Barleria prionities*, Dopamine, Norepinephrine, Serotonin, Antioxidant.

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INTRODUCTION

Barleria prionities is commonly known as porcupine flower, It is a herb, small spiny bush, 0.6-1.5 m height, much branched, bark is whitish. Leaves are 9-18 cm length, 2.5-5.7 cm wide, elliptic, acuminate, entire margin, lineolate, corolla is long and yellowish, capsules 2-2.5 cm long ovoid with a long tapering solid beak, 2-seeded. Seeds are 8 mm in diameter compressed clothed with silky appressed hairs [1]. It is a well known plant in Ayurveda. It is distributed throughout the India and many parts of the world like Ceylon USA, Astralia, Indonesia, Asia, Malaysia, Philippines [2]. Whole plant especially leaves and roots are used. Traditionally *Barleria prionities* is used in the treatment of fever, respiratory diseases, toothache and joint pains. Leaf juice of white and yellow variety is used in joint pains, as wound healing agent and mixed with cumin seeds and used in spermatorrhoea. Juice of leaves also applied to the feet in the rainy season to prevent cracking (or) laceration [2].

Paste and mouth wash are prepared from root of BP and applied to the treatment of boils, glandular swellings to relieve toothache and bleeding gums. Tooth powder is prepared from the plant and plant parts are used in the treatment of catarrh, cough and anasarea, extract of the plant was used in herbal [3] cosmetics and hair product to promote skin and scalp health. In the Konkan, the dried bark is used in the treatment of whooping cough, and juice from the fresh bark with milk act as diaphoretic and expectorant [4]. Especially the whole plant and root is much more used as a diuretic and tonic in Ceylon [3].

The plant is rich in potassium, flavonoids, iridoid glucosides and fatty acid . The plant extract was used as potent hepatoprotective agent [5-7] and also useful in the treatment of respiratory infections [8] , whooping cough and tuberculosis [9]. The root is used to relieve fever and glandular swelling and have 100% antifertility activity [10].

This plant has many uses but the antiparkinsonian potential of the plant is not yet to be explored. So *Barleria prionities* was selected for the present study as it is one of the plant in many ayurvedic formulations used for Parkinsonism.

MATERIAL AND METHODS

Collection and extraction of BP seed

Barleria prionities roots were collected locally at Thummalakunta Village, Tirupati, Chittoor District, Andhra Pradesh, India in the month of November before fruiting of plant. The collected plant material was authenticated by Botanist Dr. Madhava Chetty, Department of Botany, S.V.University, Tirupati, India. Voucher specimens of the plants were deposited in Botany Department of S.V.University, Tirupati. The plant materials were cleaned thoroughly, shade dried and crushed to a coarse powder. The powder was passed through sieve No.40 and used for further studies. Dried coarse powder of BP root was extracted with petroleum ether and then with alcohol, the yield was 12.4% (w/w).

Chemicals

MPTP (1-methyl, 4-phenyl-1, 2, 3, 6-tetrahydropyridine) was obtained from Sigma Chemical Co. USA and all other chemicals were of analytical grade.

Animals

Male Swiss albino mice weighing 25-30 g were used. They were housed in groups of five under standard laboratory conditions at temperature 23 ± 1°C relative humidity of 55 ± 5%. The animals had free access to pellet diet (Hindustan Lever foods, Bangalore, India), and water ad libitum. The animals were acclimatized to laboratory conditions for 7 days.

Study Protocol

The animals were divided into six groups, each consisting of six mice. Group-I served as vehicle control and received 2% Tween 80 (P.O), Group-II received 20mg/kg MPTP four injections (i.p) at 2 hours intervals [11], Group-III, IV, V received plant extracts at the dose of 100, 200 and 300 mg/kg (P.O) on the first day 30 minutes prior to first injection of MPTP and daily for another six days of the experimental period. Group -VI received only BP extract 300 mg/kg. At the end of experimental period (after 7 dayS of treatment) the animals were fasted overnight and sacrificed by cervical decapitation. The brains were excised immediately and the brain tissue was homogenized in ice cold butanol solution and used for further analysis.

Behaviour parameters

Test for Locomotor activity

The locomotor activity was measured using Actophotometer (Inco, Ambala, India). It consists of cage which has 30 x 30 x 30 cms and at the bottom six lights and 6 photo cells were placed in the outer periphery of the bottom in such a way that a single mice blocks only one beam. Photocell is activated when the rays of light falls on photocells, the beam of light is cut as and when animal crosses the light beam, number of cut off's were recorded for 10 minutes [12].

Test for alertness: Hole Board Test

This test was done using Hole Board. The Hole Board consisted of a $0.5m^3$ wooden board with 16 holes (3cm in diameter). The mice was placed at the corner of the board and allowed to move freely. First two minutes were allowed for adaptation and the number of head dippings in next four minutes were counted [13].

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Motor Co-ordination Test (Rota Rod Test)

Motor Co-ordination test was conducted using a Rota Rod apparatus (Inco Ambala, India). The animals were placed on the moving rod prior to the treatment and the mice stayed on the rod without falling for 120 seconds were chosen for the study. The time animals take for falling from the rotating rod was noted before and after the treatment with extract [14].

Biochemical estimations

Estimation of dopamine, epinephrine, nor-epinephrine and serotonin

Tissue samples were homogenized in ice cold butanol to give a final concentration of 50 mg/ml. The homogenates were centrifuged at 800 g for 15 minutes at 4°C. Residue was discarded and to the supernatants 2.5 ml of distilled water and 2.5ml of n-heptane were added. The contents were thoroughly mixed and centrifuged at 1000g for 5 minutes. The aqueous phase was separated and to this 200 mg of alumina was added followed by 1.5 ml of 2M sodium acetate and the PH was adjusted to 8.0 using 1N sodium hydroxide. The samples were again centrifuged at 1000xg for 5 minutes (1.5 ml supernatant was collected and used for the estimation of serotonin (5-HT) [15].

Extraction of Dopamine

The alumina was washed twice with 2ml of distilled water by vortexing the tube and centrifuged at 1000xg for 5 minutes. The supernatant was discarded and walls of the tube were blotted with strips of filter paper. The monoamines were eluted by shaking the alumina with 2ml of 2N acetic acid. The tubes were centrifuged at 100xg for 5 minutes. The supernatant was transferred to another tube. To this 100 µl of EDTA was added and the PH was adjusted to 6.3, 100µl of iodine was added to the above tube and mixed thoroughly. The samples were allowed to stand at room temperature for 2 minutes, then 200 µl of alkaline sulphite solution was added. The contents were shaken well and allowed to stand at room temperature for 2 minutes. The PH of the solution was adjusted to 5.4 with 5N acetic acid [15]. The fluorescence of epinephrine was read in a Shimadzu Spectrofluorimeter (Model No. RF 1501) with excitation and emission wavelength of 410 nm and 500 nm respectively with a band width of 10/10 nm. After reading epinephrine the same samples were heated in a boiling water- bath for 2 minutes. The tube were cooled and fluorescence of nor-epinephrine was read with excitation and emission wavelength of 385 and 485 nm respectively with slit widths of 10/10nm.The samples were again heated for 5 minutes in a boiling water bath and cooled. The fluorescence of dopamine was read at excitation and emission wavelengths of 320 and 370 nm respectively with slit widths of 10/10nm.The amine content of each sample was calculated by the method of Ansell and Beeson (1968) and the content was expressed as µg/gm wet wt of tissue.

Estimation of serotonin (5-Hydroxy tryptamine)

1.5 ml of supernatant (as described under Dopamine estimation) was taken and to this 100 µl of cysteine, 1.5 ml of hydrochloric acid and 100 µl of O-Phthaldialdehyde (OPA) solution was added. The tubes were kept at room temperature for 20 minutes. 100 µl of sodium metaperiodate was added and the tubes were heated at 80°C in a boiling water bath for 20 min. The samples were cooled and the fluorescence of serotonin was read in a Spectrofluorimeter with excitation and emission wavelength of 360 and 470 nm respectively with slit width of 20/10nm. The amount of serotonin was calculated by the method of Ansell and Beeson (1968) and expressed in µg/gm wet wt of tissue.

Estimation of Proteins

5% w/v brain homogenates were prepared in TCA (trichloroacetic acid). 0.2ml supernatant was collected and 4ml of alkaline copper sulphate was added, kept at room temperature for 20min. Then 0.4 ml of folin phenol reagent was added and kept at room temperature for 20 min. The colour developed was read at 600 nm in Spectrophotometer (Model No. ELICO, 171) [16].

Antioxidant Studies

Brain tissue was homogenized in 50 m mol phosphate buffer (PH 7.0) containing 0.1 mM ethylene diamine tetra acetic acid (EDTA) to give 5% (W/V) homogenate. The homogenate was centrifuged at 10,000 RPM for 10 min at 0°C in cold centrifuge; the resulting supernatant was used for further studies.

Lipid Peroxidation

MDA level were measured according to the method of Ohkawa et al., 1979 at room temperature. 200µl of supernatant was added to 50µl of 8.1% sodium dodecyl sulphate, vortexed and incubated for 10 min at room temperature. 375µl of thiobarbituric acid (0.6%) was added and placed in a boiling water bath for 60 min and then the sample was allowed to cool to room temperature. A mixture of 1.25 ml of butanol: Pyridine (1.5:1) was added, vortexed and centrifuged at 1000 RPM for 5 mins. The coloured layer (500 µl) was measured at 532 nm on a (ELICO,171) Spectrophotometer the values were expressed in m moles of MDA formed for mg protein/hr/or min [17].

Reduced Glutathione

Reduced glutathione levels were measured according to the method of Ellman, 1959 at room temperature. 0.75ml of supernatant was mixed with 0.75ml of 4% sulphosalicylic acid and then centrifuged at 1200 RPM for 5 min at 4°C, from this 0.5ml of supernatant was taken and added to 4.5 ml of 0.01 M DTNB and absorbance was measured at 412 nm by using a (ELICO, 171) UV-Visible Spectrophotometer [18].

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Statistical Analysis

All values were expresses as Mean ± SEM. The data of Biochemical estimations was analysed using one way (ANOVA) test for multiple comparison followed by Tukey-Krammer test using Graph pad Instat version 3. Behavioural parameters were analysed using one way ANOVA followed by Dunnet's 'T' test. In all tests, the criteria for statistical significance was $P < 0.05$.

RESULTS

Behavioural Study of Mice after MPTP treatment

Spontaneous motor activity was significantly (P<0.001) decreased in MPTP treated group as compared to control group. Locomotor activity was significantly (P<0.001) and dose dependently increased on 1st, 3rd and 6th day of treatment at given dose as compared to MPTP treated group (Table.1).

Results of Rota rod test showed significant (P<0.001) decrease of motor co-ordination in MPTP treated group as compared to control group. Retention time was reversed significantly (P<0.001) dose dependently on 1st, 3rd and 6th day of treatment with alcoholic extract of BP root as compared to MPTP treated group (Table.1).

Animals were treated with MPTP showed significantly (P<0.001) reduced alertness as compared to control vehicle group. Alertness was significantly (P<0.001) increased with alcoholic extract of BP at a dose of 200 & 300 mg/kg on 1st day and it was significantly (P<0.001) dose dependently increased with given doses on 3rd and 6th day of treatment as compared to MPTP treated group (Table.1).

Effect on dopamine level in the brain

The result of the present study revealed that the dopamine level was significantly (P<0.001) decreased in the MPTP treated animals as compared to control group and their level was significantly (P<0.001) increased at 200 & 300 mg/kg with alcoholic and aqueous extract of BP as compared to MPTP group. The petroleum ether and ethyl acetate extract of *Barleria prionities* showed significant (P< 0.01, P<0.001) increase of dopamine level at a dose of 200mg and 300 mg/kg dose as compared to MPTP treated group (Table.2).

Effect on brain epinephrine level

Epinephrine level was significantly (P<0.001) decreased in MPTP treated group as compared to control vehicle group, while their level was significantly (P<0.001) increased with alcoholic, aqueous and ethyl acetate extract at 300 mg/kg as compared to MPTP group, not significantly increased of epinephrine level with petroleum ether extract when given with MPTP. However, only extract at a dose of 300 mg/kg had significant activity as compared to MPTP group (Table.3).

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Table 1: Effect of Alcoholic extracts of *Barleria prionities* root on Spontaneous motor activity, Grip strength, Alertness (Hole Board test) in MPTP treated mice

Groups	Spontaneous motor activity score			Grip Strength in Seconds			Alertness (no. of head dippings)		
	1st day	3rd day	6th day	1st day	3rd day	6th day	1st day	3rd day	6th day
Control	446.6 ± 12.0	446.6 ± 12.0	446.6 ± 12.0	120 ± 0	120 ± 0	120 ± 0	50.5 ± 2.23	50.5 ± 2.23	50.5 ± 2.23
MPTP	278.5 ±0.7 ***	179.8 ± 5.3 ***	90.3 ± 6.2 ***	83 ± 2.8 ***	33.6 ± 7.2 ***	13.8 ± 1.6 ***	22.3 ± 1.6 ***	14.0 ± 2.1 ***	8.6 ± 1.2 ***
100mg/kg CGE + MPTP	285.8 ± 5.6 ***	292.6 ± 2.4 ***, +++	300.6 ± 1.3 ***, +++	92 ± 3.0 ***, +	91.6 ± 1.7 ***, +++	95.83 ± 1.0 ***, +++	24.8 ± 1.5 ***	31.5 ± 1.2 ***, +++	34.0 ± 1.7 ***, +++
200 mg/kg CGE + MPTP	299.1 ± 1.1 ***	302.6 ± 1.3 ***, +++	324.0 ± 3.5 ***, +++	96 ± 1.6 ***, ++	98.5 ± 0.9 ***, +++	102.1 ± 3.3 ***, +++	31.0 ± 1.1 ***, ++	35.8 ± 1.1 ***, +++	37.5 ± 0.5 ***, +++
300 mg/kg CGE + MPTP	303.1 ± 1.8 ***, +++	328.1 ± 3.1 ***, +++	436.8 ± 2.3 +++	100 ± 1.0 ***, +++	101 ± 0.7 **, +++	14.3 ± 1.4 ***, +++	31.6 ± 1.1 ***, ++	38.6 ± 1.2 ***, +++	39.6 ± 1.1 ***, +++
300 mg/kg CGE	317.0 ± 3.1 ***, +++	336.8 ± 3.0 ***, +++	455.5 ± 17.4 +++	101 ± 2.2 ***, +++	106.8 ± 1.4 +++	111.6 ± 0.8 **, +++	46.8 ± 1.6 +++	48.6 ± 1.0 +++	52.0 ± 1.8 +++

Values are expressed as Mean ± SEM (n = 6);
** (P<0.01), *** (P< 0.001) Vs Control group; + (P< 0.05), ++ (P< 0.01), +++ (P< 0.001) Vs MPTP group.

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Table 2: Effect of *Barleria prionities* root on brain dopamine level in MPTP pretreated mice.

Groups	µg/g brain tissue			
	Alcoholic extract	Petro. ether fraction	Ethyl acetate fraction	Water extract
Control	3.815 ± 0.055	3.815 ± 0.055	3.815 ± 0.55	3.815 ± 0.055
MPTP	1.257 ± 0.038***	1.257 ± 0.038***	1.257 ± 0.038***	1.257 ± 0.038***
100mg/kg BPE+ MPTP	1.516 ± 0.12***	1.280 ± 0.042***	1.317 ± 059***	1.399 ±0.049***
200 mg/kg BPE + MPTP	1.926 ± 0.11*** +++	1.585 ± 0.044*** ++	1.895± 0.14*** ++	1.901 ± 0.036*** +++
300 mg/kg BPE + MPTP	2.915 ± 0.09 *** +++	2.465 ± 0.093*** +++	2.875 ± 0.050*** +++	2.922 ± 0.032*** +++
300 mg/kg BPE	3.088 ± 0.12*** +++	2.749 ± 0.039*** +++	3.006 ± 0.024*** +++	3.015 ± 0.029*** +++

Values are expressed as Mean ± SEM (n = 6); *** (P<0.001) Vs Control group; ++ (P< 0.01), +++ (P< 0.001) Vs MPTP group.

Table 3:Effect of *Barleria prionities* root on brain epinephrine level in MPTP treated mice.

Groups	µg/g brain tissue			
	Alcoholic extract	Petro.ether fraction	Ethyl acetate fraction	Water extract
Control	3.288 ± 0.102	3.288 ± 0.102	3.288 ± 0.102	3.288 ± 0.102
MPTP	2.106± 0.090***	2.106 ± 0.090***	2.106 ± 0.090***	2.106 ± 0.090***
100mg/kg BPE + MPTP	2.213± 0.068***	2.131 ± 0.154***	2.151 ± 0.46***	2.192 ± 0.079***
200 mg/kg BPE + MPTP	2.264 ± 0.062***	2.198 ± 0.104***	2.116 ± 0.111***	2.251 ± 0.040***
300 mg/kg BPE + MPTP	2.635 ± 0.045*** +++	2.214 ±0.038***	2.670 ± 0.038 ***,+++	2.738 ± 0.043 ***, +++
300 mg/kg BPE	3.208 ± 0.137+++	2.913 ± 0.054+++	3.035 ± 0.065+++	3.100 ± 0.083+++

Values are expressed as Mean ± SEM (n = 6); *** (P<0.001) Vs Control group; +++ (P<0.001) Vs MPTP group.

Effect on brain norepinephrine level

Results of the present study showed that nor-epinephrine level was significantly (P<0.001) altered by MPTP treated group, while it was significantly increased at a dose of 300 mg/kg with alcoholic, aqueous and ethyl acetate extract of BP as compared to MPTP group. Plant extract alone at a dose of 300 mg/kg significantly increased serotonin level as compared to MPTP treated group (Table.4).

Effect on brain serotonin level

The serotonin level was significantly (P<0.001) reduced in MPTP treated mice, while its level was restored significantly (P<0.01, 0.001) at a dose of 200, 300 mg/kg with alcoholic and aqueous extract, while ethyl acetate extract showed significant (P< 0.001) enhance of serotonin at dose of 300 mg/kg as compared to MPTP treated group. Petroleum ether had no significant

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results in combination with MPTP but it showed significant (P<0.001) improvement at a dose of 300 mg/kg plant extract alone as compared to MPTP group (Table.5).

Table 4: Effect of *Barleria prionities* root on brain norepinephrine level in MPTP pretreated mice.

Groups	µg/g brain tissue			
	Alcoholic extract	Petro.ether fraction	Ethyl acetate fraction	Water extract
Control	3.343 ± 0.054	3.343 ± 0.054	3.343 ± 0.054	3.343 ± 0.054
MPTP	2.148 ± 0.041 ***	2.148 ± 0.41 ***	2.148 ± 0.041 ***	2.148 ± 0.041 ***
100mg/kg BPE + MPTP	2.214 ± 0.070 ***	2.164 ± 0.071***	2.168 ± 0.046 ***	2.190 ± 0.037 ***
200 mg/kg BPE + MPTP	2.289 ± 0.125 ***	2.170 ± 0.082 ***	2.184 0.054 ***	2.205 ± 0.054 ***
300 mg/kg BPE + MPTP	2.850 ± 0.044 ***,+++	2.202 ± 0.037 ***	2.703 ± 0.088 ***,+++	2.731 ± 0.083 ** +++
300 mg/kg BPE	3.293 ± 0.157 +++	3.028 ± 0.046 +++	3.158 ± 0.058+++	3.173 ± 0.055 +++

Values are expressed as Mean ± SEM (n = 6); ** (P<0.001), *** (P<0.001) Vs Control group; +++ (P<0.001) Vs MPTP group.

Table 5: Effect of *Barleria prionities* root on brain serotonin level in MPTP treated mice.

Groups	µg/g brain tissue			
	Alcoholic extract	Petro. ether fraction	Ethyl acetate fraction	Water extract
Control	3.407 ± 0.050	3.407 ± 0.050	3.407 ± 0.050	3.407 ± 0.050
MPTP	2.068 ± 0.060 ***	2.068 ± 0.060 ***	2.068 ± 0.060 ***	2.068 ± 0.060 ***
100mg/kg BPE + MPTP	2.411 ± 0.091 ***	2.125 ± 0.047 ***	2.219 ± 0.162 ***	2.221 ± 0.085 ***
200 mg/kg BPE + MPTP	2.508 ±0.284 **, ++	2.227 ± 0.100 ***	2.323 ± 0.047 ***	2.469 ± 0.119 ***, +
300 mg/kg BPE + MPTP	2.616 ± 0.084 **,+++	2.344 ± 0.068 ***	2.783 ± 0.04 *** +++	2.515 ± 0.057 ***, ++
300 mg/kg BPE	3.321 ± 0.134 +++	2.969 ± 0.090 ** +++	3.038 ±0.035 * +++	3.106 ± 0.055 +++

Values are expressed as Mean ± SEM (n = 6); * (P< 0.05), ** (P<0.01), *** (P< 0.001) Vs Control group ; + (P< 0.05), ++ (P< 0.01), +++ (P< 0.001) Vs MPTP group.

Antioxidant studies

Effect on Lipid peroxidation

MDA level was significantly (P<0.001) increased in the MPTP treated group as compared to control vehicle group. On treatment with alcoholic extract of BP significantly (P<0.001) dose dependently decreased the MDA level as compared to MPTP treated animals. Animals treated

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with aqueous and ethyl acetate extract in combination with MPTP revealed the significant (P< 0.01, 0.001) decrease of MDA level at 200, 300 mg/kg dose as compared to MPTP group. Petroleum ether extract of BP exhibited significant (P<0.01) decrease of MDA level at a dose of 300 mg/kg as compared to MPTP treated group (Table.6).

Table 6: Effect of *Barleria prionities* root on Lipid peroxidation (nmol/mg protein/hr) level in MPTP treated mice.

Groups	nmol/mg protein/hr			
	Alcoholic extract	Petro. ether fraction	Ethyl acetate fraction	Water extract
Control	26.25 ± 3.4	26.25 ± 3.4	26.25 ± 3.4	26.25 ± 3.4
MPTP	67.0 ± 5.6 ***	67.0 ± 5.6 ***	67.0 ± 5.6 ***	67.0 ± 5.6 ***
100mg/kg BPE + MPTP	44.83 ± 3.3 ** +++	61.58 ± 1.9 ***	59.20 ± 2.2 ***	56.18 ± 2.2 ***
200 mg/kg BPE + MPTP	33.5 ± 1.9 +++	56.68 ± 2.2 ***	45.25 ± 3.8 ** +++	43.83 ± 3.0 ** +++
300 mg/kg BPE + MPTP	27.3 ± 1.4 +++	45.00 ± 3.3 * ++	35.29 ± 2.8 +++	35.29 ± 2.1 +++
300 mg/kg BPE	22.7 ± 2.1 +++	31.83 ± 1.6 +++	29.55 ± 0.5 +++	28.02± 0.8 +++

Values are expressed as Mean ± SEM (n = 6); * (P< 0.05), ** (P< 0.01), *** (P< 0.001) Vs Control group; ++ (P< 0.01), +++ (P< 0.001) Vs MPTP group.

Effect on Reduced glutathione level

Reduced glutathione level was significantly (P<0.001) reduced in MPTP treated group as compared to control group. GSH level was restored significantly (P<0.05, 0.01) with alcoholic and aqueous extract at a dose of 200, 300 mg/kg as compared to MPTP treated group. Petroleum ether of BP when given in combination with MPTP showed moderately significant (P<0.05) increase of GSH level at 300 mg/kg as compared to MPTP treated group. Ethyl acetate extract exhibited significant (P<0.01) effect on GSH level at 300 mg/kg as compared to MPTP treated animals (Table.7).

Table 7: Effect of *Barleria prionities* root on reduced glutathione (µmol/mg protein) level in MPTP pretreated mice.

Groups	µmol/mg protein			
	Alcoholic extract	Petro. ether fraction	Ethyl acetate fraction	Water extract
Control	759.90 ± 53.2	759.90 ± 53.2	759.90 ± 53.2	759.90 ± 53.2
MPTP	476.60 ± 24.9 ***	476.60 ± 24.9 ***	476.60 ± 24.9 ***	476.60 ± 24.9 ***
100mg/kg BPE + MPTP	544.33 ± 24.9 ***	484.43 ± 22.1 ***	496.4 ± 4.6 ***	542.87 ± 18.6 ***
200 mg/kg BPE + MPTP	616.73 ± 16.5 * +	494.43 ± 6.1 ***	548.97 ± 20.9 ***	609.17 ± 13.2 **, +
300 mg/kg BPE + MPTP	676.10 ± 15.8 ++	597.90 ± 6.5 **, +	631.35 ± 16.4 * ++	666.10 ± 16.3 +++
300 mg/kg BPE	721.17 ± 20.9 +++	709.20 ± 15.1 +++	729.17 ± 4.8 +++	729.53 ± 4.6 +++

Values are expressed as Mean ± SEM (n = 6); * (P< 0.05), ** (P< 0.01), *** (P< 0.001) Vs Control group; + (P< 0.05), ++ (P<0.01), +++ (P< 0.001) Vs MPTP group.

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DISCUSSION

The present study revealed the quantitative behavioural responses with in a short span of seven days in MPTP lesioned animals. The treated animals were subjected to the spontaneous motor activity, rota rod test, and exploratory behaviour. Decreased SMA could be due to motor impairment, retention time was also decreased it could be due to loss of muscular strength. Number of head dipping were also reduced, it might be due to motor impairment. Behavioral complications altered by MPTP treatment were reversed with alcoholic extract of BP root. Maximum protection of extract was observed on 6^{th} day of treatment, it might be due to lag time required for restoration.

Dopamine depletion was also occured in MPTP treated mice. Altered behavioral responses followed by DA depletion are similar to human Parkinsonism [19-21].

Dopamine neurotransmitter was more affected in Parkinson's disease [22-23] where as other brain amines like norepinephrine, epinephrine and serotonin were much less affected than dopamine in MPTP treated group [24]. Norepinephrine was less altered in parkinsonian patient this is in accordence with earlier reported studies [25]. Altered norepinephrine might contribute to some aspects of intellectual dysfunction in PD [26].

Dopamine is the precursor to norepinephrine, norepinephrine is a precursor to the hormone epinephrine. Norepinephrine and epinephrine are antistress chemicals in the body, obviously there is great stress from Parkinson's disease. Further, epinephrine is involved in increasing the power of muscles and prolonging the action of muscle, by its ability to activate the release of glucose from glycogen. Thus optimizing the ability of epinephrine may help achieve more muscle control, perhaps reducing motor symptoms of Parkinson's disease [27].

Depression is a common symptom in patients with Parkinson's disease. Alterations in serotonin metabolism are found in primary depression. The brain content of serotonin in Parkinson's disease is also reduced, but this has not been related to any manifestation of the disorder. Cerebrospinal fluid (CSF) content of the major metabolite of serotonin, 5- hydroxyl indole acetic acid was lower in depressed than non depressed Parkinsonian's patients, suggest that the alterations in serotonin metabolism in Parkinson's disease identify a subgroup of patients who are prone to depression [28]. DA and 5-HT transporters are differentially affected in PD and 5-HT transporters in the mid brain region may not be affected in relatively early stages of PD [29].

The loss of dopamine and other amines was reversed by alcoholic and aqueous fractions of BP in a dose dependent manner. In the tested fractions alcoholic fraction showed maximum neuroprotection. It could be due to presence of more alcohol soluble components like flavonoids, proteins and L-dopa. Alcohol and water are good solvents for extraction of L-dopa [30]. Antiparkinsonian effect of BP extract not only due to presence of L-dopa but also other compounds. Reactive oxygen species can be eliminated by a enzymatic and non enzymatic

antioxidants and thus protecting tissue/ organ damage from oxidative stress. In the present study, we estimated non enzymatic antioxidants and brain lipid peroxidation *in vivo*.

Malondialdehyde (MDA) is an end product of lipid peroxidation, a non enzymatic antioxidant present in less concentration scavenges hydroxyl free radicals [31]. In our study, increase of MDA level was observed in MPTP treated mice than in the group treated with plant extract. These findings are similar to earlier reports [32] on *Boerhavia diffusa* Linn). MDA level was decreased with alcoholic and aqueous fractions of BP root. Glutathione is a nonenzymatic antioxidant. Glutathione peroxidase a selenium containing enzyme present in significant concentrations detoxifies hydrogen peroxide to water through the oxidation of reduced glutathione [33]. Decreased level of reduced glutathione was observed in MPTP treated mice. GSH level was restored with alcoholic and aqueous fractions of root. Medicinal herbs with antioxidants are useful in diseases in which free radicals are involved such as, anoxia, ischemia of Parkinsonism, arteriosclerosis, rheumatism and cancer [34-38]. Several studies on medicinal plants with free radical scavenging and antioxidant activities indicate that these activities are due to presence of poly phenols and flavonoids in aqueous fraction [38] and preliminary phytochemical test of BP indicated that the presence of phenolic compounds and flavonoids. Hence, the antioxidants and free radical scavenging activity of the plant may be due to presence of these compounds.

CONCLUSION

Barleria prionities exhibits dopaminergic neuroprotection in MPTP induced parkinsonism in mice. It prevents MPTP induced behavioral alterations and dopaminergic neuronal loss. Hence, *Barleria prionities* can exert a significant neuroprotective effect and its usefulness in the management of Parkinson's disease needs further exploration to identify the mechanism and active phytoconstituents.

REFERENCES

[1] Kirtikar KR and Basu BD. In: Indian Medicinal Plants, Vol I, 2nd ed, International Book Distributors, Dehradun, India, 1995; pp. 790-791.
[2] Burkill HM. The useful plants of west Tropical Africa, UK, Royal Botanic Garden, Kew, 1985; pp.1960.
[3] Nadakarni AK. In: Indian Materia Medica, Vol I, Popular Prakashan Pvt.Ltd, Bombay, 1976; pp. 175-177.
[4] Gupta HM and Saxena VK. Bulletin of Medico-Ethno Botanical Research 1984; 5(3): 178-83.
[5] Gujral ML, Saxena PN, Mishra SS. J Indian Med Assoc 1995; 25: 49.
[6] Nagarjun S, Barnabas CGG. Fitoterapia 1986; 59(6): 508-510.
[7] Singh B, Chandan BK, Prabhakar A, Taneja SC, Singh J, Qazi GN. Phytother Res 2005; 18: 391-404.

[8] Chen JL, Blanc P, Stoddart CA, Bogan M, Rozhon EJ, Parkinson N. J Nat Prod 1998; 612: 1295-7.
[9] Oomachan MM. J Pure and Appli Sci 1991; 6: 39- 43.
[10] Gupta RS, Kumar P, Dixit VP, Dobhal MP. J Ethnopharmacol 2000; 70: 111-7.
[11] Lewis JV, Jacowec M, Burke RE, Przedborski S. Neurodegenration 1995; 4:257-69.
[12] Goyal RK. Practical in Pharmacology, 5th ed, B.S.Shah Prakashan, Ahmedabad, 2005; pp.121-122.
[13] File SE and Wardril AG. Psychopharmacol 1975; 44: 53-59.
[14] Kulkarni SK. Hand book of experimental pharmacology, Vallabh Prakashan, New Delhi, 1987; 122.
[15] Kari HP, Dvidson PP, Herbert HH, Kochbar MH. Res Comm Chem Path Pharmacol 1978; 20: 475-488.
[16] Lowry OH, Rose brough NJ, Farr AL, Randall RJ. J Biol-chem 1951; 193: 265-275
[17] Ohkowa N, Ohishi H, Yagi. Anal Biochem 1979; 95: 331-335.
[18] Ellman GL. Arch Biochem Biophys 1959; 82: 70-77.
[19] Cousin MS and Salamone JD. 1996. Brain.Res 1996; 732: 186-94.
[20] German DC, Nelson EC, Liang CL, Speciale SG, Sinton CM, Sonsalla PK. Neurodegeneration 1996; 5: 299-12.
[21] Pisa M. Neuro Pharmacol Biol Psy 1998; 12: 217-24.
[22] Kish PE, Fisher BA, Ueda T. Natl Acad Sci USA 1989; 86: 3877-3881.
[23] Horniykiwicz O. Neurol 1960; 45: 19-34.
[24] Robert SF, Jerrold SM, Linda FQ. 1987. Principles of Neuropsycho-pharmacology, Sinauer Associates Inc Publishers, Sunderland, 1987; pp. 861-886.
[25] Stephen J, Kish PD, Kathleen S, Shannak, Ali H, Rajput MD, Joseph, Gilbert MD, Oleh Hornykiewicz MD. Arch Neurol 1984; 41(6): 612-614.
[26] Yaadov S, Richard MD, Lucien CMD. Neurology 1984; 41(10): 10.
[27] Bonnet AM and Houeto JL. J Biomed and Pharmacother 1999; 53:117-121.
[28] Mayeux R, Stern Y, Cote L, William J. Neurology 1984; 34 (5): 642.
[29] Sang EK, JOO YC, Yearn SC, Yong C, Won YL. Journal of Nuclear Medicine 2003; 44(6): 870-876.
[30] Laxminarain M and Hildebert W. Indian journal of Biochemistry and Biophysics 2007; 44: 56-60.
[31] Auddy B, Ferreiru M, Blasina F, Lafon L, Arredondo F and Dajas F. J Ethanopharmacol 2003; 84: 131-138.
[32] Amarnath PL. J Ethnopharmacol 2004; 91: 109-113.
[33] Bruce A, Freeman D and James C. Lab Invest 1982; 47: 412-426.
[34] E1-Tahir KE, Ashour MM, A1-Harbi MM. General Pharmacol 1993; 24: 1123-31.
[35] Houghton PJ, Zarka R, Dela Heras B, Hout JRS. Planta Medica 1995; 61: 33-36.
[36] Medicana R, Janssens J and Tarasenko A. Cancer Res 1997; 38: A1377.
[37] Badary OA, Abdel-Niam AB, Abdel-Wahab MH, Hamada FM. Toxicol 2000; 143: 219-26.
[38] Al Ghamdi MS. J Ethnopharmacol 2001; 76: 45-48.
[39] LU Y and FOO LY. Food Chem 2001; 75: 197-202.