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Neuroprotective effect of various extracts of *Prosopis chilensis* in MPTP induced neurotoxicity in mice

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ABSTRACT

The aim of the study was to evaluate neuroprotective effect of various (alcoholic, petroleum, ethyl acetate and aqueous) extracts of Prosopis chilensis (PC) seeds in MPTP (1-methyl,4-phenyl-1,2,3,6-tetra hydro pyridine) mouse model. PC seed 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 (20 mg/kg/i.p) 4 injections at 2 hours intervals. Behavioural parameters were assessed on 1st, 3rd and 6th 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 extract of PC significantly (P< 0.01, 0.001) and ethyl acetate, aqueous extracts significantly (P< 0.001) improved the brain dopamine and other amines like norepinephrine, epinephrine and serotonin at a dose of 200 and 300 mg/kg and also significantly (P< 0.001) improved the reduced glutathione level and decreased the melondialdehyde (MDA) level as compared to MPTP control group, while petroleum ether extract has no significant effect on dopamine and other amines when combine with MPTP. However, the alcoholic extract showed maximum activity while ethyl acetate and aqueous extract showed similar activity. Hence, PC placed a most important plant due to presence of L-dopa, poly phenols, amino acids and potential antioxidant activity. These results indicating evidence for PC extract had a neuroprotective effect on MPTP induced Parkinsons.

Key words: Antiparkinson, L-dopa, *Prosopis chilensis*, MPTP, Antioxidants.

INTRODUCTION

Prosopis chilensis (Mol.) Stuntz also known as algarrobo belongs to leguminosea. It is a native tree extending from Peru and Boliva to central chile and north western Argentina. It is a tree with

short trunk, 3-10 m tall, tree top is rounded branchlets flexuous, partly spinous, spines on strong shoots. Leaves are deciduous and glabrous. Flower is greenish white to yellowish, legumes are linear compressed with parallel margin, straw yellow coloured, acuminate, nearly straight, thick, mesocarp is sugary edible, seeds are ovoid with bean shaped, oblong compressed and brown coloured [1]. *Prosopis* pod is a modern food source and has been suggested by several recent chemical and nutritional studies [2-4]. The whole pod contains 11-17% protein and 13-34% sugar with the protein being concentrated in the seed (26-37% of seed) seeds contains small quantity of saponins. *Prosipis* contains apigenin-8-glucoside, apigenin-6-glucoside, quercetin-3-glucoside, quercetin-3-rhamnoside, quercetin-3-rutinoside and traces of myricetin-3-rhamnoside, luteolin, kaempferol-3-omequercetin [5]. Bark and roots contain tannins, young leaves contain 1.8%, intermediate leaves contain 1.7% and mature leaves contain 0.9% of alkaloids [5]. The protein rich seed cotyledon was separated as fraction and found to have uses like typical of bean proteins. This protein is nutritionally limiting in tyrosine and methionin/cysteine [6]. Aerial parts of *Prosopis chilensis* contain tryptamine, beta-phenethylamine and its derivatives [7]. This species has economic importance due to its high quality of the timber and has been recognized as valuable for reforestation in the worlds of semi arid regions. It is also utilized as a protein source for live stock. Pods are eaten as sweets “patay” or drunk as “aloja”. It is a staple food for cattle in arid regions [1]. Africans believe that the ripe pods are make excellent fodder but the green pods are bitter and value less, reddish mesquite gum may be used as a substitute for *Gum arabica* as an adhesive and also used in the manufacturing of gum drops [8], and Infusion prepared from the leaves showed some antibiotic activity [9].

EXPERIMENTAL SECTION

Collection and extraction of PC seed:

Prosopis chilensis pods were collected in Sri Padmavathi Visvavidyalayam campus, Tirupati, Chittoor district, Andhra Pradesh, India in the month of April. The collected plant was authenticated by Botanist Dr. Madhava Chetty, Department of Botany, S.V.University, Tirupati, Andhra Pradesh, India. Voucher specimen (No.2510) of the plants were deposited in Botany Department of S.V.University, Tirupati. The plant materials were cleaned thoroughly, shade dried, seeds were separated from pods and crushed to a coarse powder. The powder was passed through sieve No.40 and used for further studies. Dried coarse powder of PC seed was extracted with petroleum ether and then with alcohol and yield was 15.5% (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 i.p MPTP four injections at 2 h intervals [10]. Groups 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 PC 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 interrupted as and when animal crosses the light beam, number of cut interruptions were recorded for 10 minutes [11].

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 dipping in next four minutes were counted [12].

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 [13].

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) [14].

Extraction of Dopamine:

The alumina was washed twice with 2ml of distilled water by vortexing the tube and centrifuged at 1000g 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 ethylene diamine tetra acetic acid (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.[17] The fluorescence of epinephrine (E) 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 (NE) 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 (DA) 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):
To 1.5 ml of supernatant, 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 (trichloro acetic 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 [15].

Antioxidant Studies:
Brain tissue was homogenized in 50 mmol phosphate buffer (PH 7.0) containing 0.1 mM of 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 was measured according to the method of Ohkawa et al., [16] 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**.**

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 5,5-dithiobis-(2-nitrobenzoic acid (DTNB) and absorbance was measured at 412 nm by using a (ELICO, 171) UV-Visible Spectrophotometer [17].

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

RESULTS

Acute toxicity and gross behaviour changes:

Alcoholic extracts and its fractions of *Prosopis chilensis* was found to be safe since no animal died even at the maximum single dose of 3200 mg/kg orally. The animal did not show any gross behaviour changes at the doses tested.

Anti-parkinsonian activity:

Evaluation of behavioural activity in MPTP treated animals:

Results of the present study showed that significantly (P<0.01) decreased spontaneous motor activity in MPTP treated animals when compared to control group. Alcoholic extract of PC showed no significant effect on 1st day of treatment but locomotor activity was significantly (P<0.01) dose dependently increased on 3rd & 6th day of treatment as compared to MPTP treated group **Table 1**.

Retention time was significantly ((P<0.001) decreased in MPTP treated group. Retention time was significantly (P<0.001) enhanced with AEPC on 3rd and 6th of treatment as compared to MPTP group **Table 1**.

Results of the hole board test revealed that the number of head dipping were significantly (P<0.001) reduced in MPTP treated group as compared to control group. It was significantly (P<0.001) dose dependently increased on 3rd and 6th day of treatment with alcoholic extract as compared to MPTP treated group **Table 1**.

Table 1: Effect of Alcoholic extracts of *Prosopis chilensis* seed 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 ± 2.2	50.5± 2.2	50.5 ± 2.2
MPTP	278.5 ± 7.0 ***	179.8 ± 5.3 ***	90.3 ± 6.2 ***	83.8 ± 2.8 ***	33.6 ± 7.2 ***	13.8 ± 1.1 ***	22.3 ± 1.6 ***	14.0 ± 2.1 ***	8.6± 1.2 ***
100mg/kg PCE + MPTP	296.3 ± 1.4 ***	290.1 ± 3.6 ***, +++	296.1 ± 2.2 ***, +++	83.8 ± 1.7 ***	89.6 ± 1.6 ***, +++	94.0 ± 1.7 ***, +++	23.1 ± 1.3 ***	28.8 ± 1.0 ***, +++	32.0 ± 1.6 ***, +++
200 mg/kg PCE + MPTP	299± 0.5 ***	297.0± 1.8 ***, +++	325.0 ± 4.4 ***, +++	93.0 ± 2.6 ***, +	97.5 ± 0.9 ***, +++	99.1 ± 1.0 ***, +++	29.8 ± 6.8 ***, +	33.5± 0.8 ***, +++	34.5 ± 1.3 ***, +++
300 mg/kg PCE + MPTP	300.8± 2.6 ***	326.3 ± 3.1 ***, +++	419.6± 7.5 ***, +++	97.0 ± 1.5 ***, +++	98.8 ± 1.0 ***, +++	103 ± 1.0 ***, +++	30.8±1.5 ***, +	36.1 ± 0.4 ***, +++	38.1± 1.8 ***, +++
300 mg/kg PCE	326.8 ± 3.6 ***,+++	338.8 ± 2.9 ***, +++	39.8 ± 7.9 +++	99.8 ± 1.7 ***, +++	105.8 ± 1.7 *, +++	109 ± 2.1 ***, +++	43.6 ± 2.3 +++	45.8 ± 1.5 +++	49.5± 1.7 +++

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

Effect on brain dopamine level:

Dopamine level was significantly (P<0.001) reduced in MPTP treated animals when compared to control group. Its level was significantly (P<0.001) enhanced with the alcoholic extract at 200 and 300 mg/kg as compared to MPTP group. Animals treated with pet. ether extract of *Prosopis chilensis* exhibited no significant improvement when given in combination with MPTP but improvement was observed only with plant extract. Dopamine level was significantly (P<0.001) increased with aqueous and ethyl acetate extract of PC at 300 mg/kg as compared to MPTP treated group **Table 2.**

Table 2: Effect of *Prosopis chilensis* seeds on brain dopamine level in MPTP treated mice

Groups	µg/g brain tissue			
	Alcoholic extract	**Petroleum ether extract**	**Ethyl acetate extract**	**Aqueous extract**
Control	3.815 ± 0.055	3.815 ± 0.055	3.815 ± 0.055	3.815 ± 0.055
MPTP	1.257 ± 0.038 ***	1.257 ± 0.038 ***	1.257 ± 0.038 ***	1.257 ± 0.038 ***
100mg/kg PCE	1.376 ± 0.058 ***	1.260 ± 0.018 ***	1.272± 0.053 ***	1.264 ± 0.017 ***
200 mg/kg PCE	1.582 ± 0.041 *** ++	1.268 ± 0.024 ***	1.286 ± 0.043 ***	1.286 ± 0.042 ***
300 mg/kg PCE	1.802 ± 0.040 *** +++	1.441 ± 0.034 ***	1.758 ± 0.025 *** +++	1.685 ± 0.012*** +++
300 mg/kg PCE	2.824 ± 0.065 *** +++	1.948 ± 0.070*** +++	2.794 ± 0.054 *** +++	2.713 ± 0.089 *** +++

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

Effect on brain epinephrine level:

Epinephrine level was significantly (P<0.001) altered in MPTP treated group. Mice treated with alcoholic, ethyl acetate and aqueous extract of PC showed significant (P<0.01) enhancement of epinephrine level at a dose of 300 mg/kg as compared to MPTP group. Animals treated with petroleum ether extract showed no significant improvement of epinephrine level when compared to MPTP treated group **Table 3**.

Table 3: Effect of *Prosopis chilensis* seed on brain epinephrine level in MPTP treated mice

Groups	µg/g brain tissue			
	Alcoholic extract	**Petroleum ether extract**	**Ethyl acetate extract**	**Aqueous 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 PCE + MPTP	2.210 ± 0.283 **	2.116 ± 0.062 ***	2.167 ± 0.191 ***	2.190 ± 0.165 ***
200 mg/kg PCE + MPTP	2.252 ± 0.038 ***	2.155 ± 0.094 ***	2.185 ± 0.144 ***	2.241 ±0.115 ***
300 mg/kg PCE +MPTP	2.705 ± 0.059 *** +++	2.260 ± 0.123 ***	2.606 ± 0.039 *** +	2.644 ± 0.023 *** +
300 mg/kg PCE	3.055 ± 0.109 +++	2.884 ± 0.093 * +++	3.081 ± 0.059 +++	3.096 ± 0.035+++

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

Effect on brain norepinephrine level:

Norepinephrine level was significantly (P<0.001) changed in MPTP treated animals as compared to control group. Norepinephrine level was normalized significantly (P<0.05) with alcoholic aqueous and ethyl acetate extract of *Prosopis chilensis* as compared to MPTP group. Treatment with petroleum ether extract of PC showed no significant improvement of norepinephrine level when compared to MPTP treated group **Table 4.**

Table 4: Effect of *Prosopis chilensis* seed on brain norepinephrine level in MPTP treated mice

Groups	µg/g brain tissue			
	Alcoholic extract	**Petroleum ether extract**	**Ethyl acetate extract**	**Aqueous extract**
Control	3.344 ± 0.054	3.343 ± 0.054	3.343 ± 0.054	3.343 ± 0.054
MPTP	2.148 ± 0.041 ***	2.148 ± 0.041 ***	2.148 ± 0.041 ***	2.148 ± 0.041 ***
100mg/kg PCE + MPTP	2.186 ± 0.048 ***	2.152 ± 0.052 ***	2.151 ± 0.042 ***	2.172 ± 0.122***
200 mg/kg PCE + MPTP	2.254 ± 0.094 ***	2.209 ± 0.065 ***	2.152 ± 0.047 ***	2.250 ± 0.085 ***
300 mg/kg PCE +MPTP	2.636 ± 0.092 *** +++	2.228 ± 0.075 ***	2.586 ± 0.031 *** ++	2.633 ±0.047 ***+++
300 mg/kg PCE	3.136 ± 0.078 +++	2.817 ±0.051 *** +++	3.061 ± 0.065 ** +++	3.089 ± 0.054+++

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

Effect on brain serotonin level:

Serotonin level was significantly (P<0.001) reduced in MPTP treated mice as compared to control group. Alcoholic and aqueous extract in combination with MPTP showed significant (P<0.001) improvement of serotonin level as compared to MPTP group. Treatment with petroleum ether and ethyl acetate extract of PC in combination with MPTP did not show significant improvement of serotonin level but when given only plant extract it was effective significantly (P<0.001) at a dose of 300 mg/kg as compared to MPTP treated group **Table 5.**

Table 5: Effect of *Prosopis chilensis* seed on brain serotonin level in MPTP treated mice

Groups	µg/g brain tissue			
	Alcoholic extract	Petroleum ether extract	Ethyl acetate extract	Aqueous 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 PCE + MPTP	2360 ± 0.038 ***	2.083 ± 0.023 ***	2.198 ± 0.073 ***	2.198 ± 0.066 ***
200 mg/kg PCE + MPTP	2.418 ± 0.051 *** ++	0.193 ± 0.047 ***	2.323 ± 0.090 ***	2.406 ± 0.062 *** ++
300 mg/kg PCE +MPTP	2.571 ± 0.118 *** +++	2.245 ± 0.067 ***	2.374 ± 0.074 ***	2.489 ± 0.043*** +++
300 mg/kg PCE	3.103 ± 0.039 * +++	2.790 ± 0.034 *** +++	3.088 ± 0.055 +++	3.115 ± 0.047 * +++

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

Antioxidant studies:

Effect on Lipid peroxidation:

Results of the present study showed that the brain MDA level was significantly (P<0.001) increased in MPTP treated animals as compared to control group. Alcoholic, ethyl acetate and petroleum ether extract of PC showed significantly (P<0.001) dose dependently reduction of MDA level as compared to MPTP treated group. Petroleum ether extract showed significant (P<0.01) decrease of MDA level at a dose of 300 mg/kg as compared to MPTP group **Table 6.**

Table 6: Effect of *Prosopis chilensis* seed on lipid peroxidation (nmol/mg protein/hr) level in MPTP treated mice

Groups	nmol/mg protein/hr			
	Alcoholic extract	Petroleum ether Extract	Ethyl acetate extract	Aqueous 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 PCE + MPTP	51.41 ± 1.0 *** ++	61.41 ± 0.9 ***	53.41 ± 1.5 ***	57.16 ± 0.6 ***
200 mg/kg PCE + MPTP	40.5 ± 1.9 ** +++	57.83 ± 1.0 *** ++	47.83 ± 0.7 *** ++	53.20 ± 0.9 *** +
300 mg/kg PCE +MPTP	29.9 ± 1. +++	49.79 ± 1.7 +++	37.08 ± 3.4 +++	39.62 ± 1.5 * +++
300 mg/kg PCE	20.8 ± 1. +++	32.95 ± 1.0 +++	27.50 ± 1.9 +++	31.58 ± 1.1 +++

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.

Effect on reduced glutathione level:

Present study showed the reduced glutathione level was significantly decreased (P<0.001) in MPTP treated mice as compared to control vehicle group. GSH level was restored significantly (P<0.001) with alcoholic extract of PC as compared to MPTP group. Treatment with petroleum ether extract showed no significant effect on GSH level. Ethyl acetate and aqueous extract of PC exhibited significantly (P<0.05) increased GSH level at 300 mg/kg as compared to MPTP treated group **Table 7**.

Table 7: Effect of *Prosopis chilensis* seed on reduced glutathione (µmol/mg protein) level in MPTP treated mice

Groups	µmol/mg protein			
	Alcoholic extract	**Petroleum ether extract**	**Ethyl acetate extract**	**Aqueous 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 PCE + MPTP	493.77 ± 23.6 ***	483.07 ± 11.7 ***	485.83 ± 26.2 ***	484.32 ± 25.4 ***
200 mg/kg PCE + MPTP	605.50 ± 16.0 ** +	495.37 ± 15.3 ***	573.23 ± 26.9 **	553.60 ± 22.0 ***
300 mg/kg PCE +MPTP	687.60 ± 10.4 +++	581.60 ± 9.4 ***	621.97 ± 9.8 * +	606.70 ± 24.2 ** +
300 mg/kg PCE	734.50 ± 5.3 +++	700.77 ± 23.0 +++	726.52 ± 9.4 +++	730.75 ± 7.7 +++

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

DISCUSSION

Alcoholic extract of PC assessed for the neuropharmacological evaluation in MPTP treated mice. Spontaneous motor activity, exploratory behaviour and motor coordination were decreased in MPTP treated group, it could be due to motor impairment and muscle relaxant effect. On treatment with alcoholic extract of *Prosopis chilensis* reversed the behavior alterations induced by MPTP on 3rd day, but maximum effect of extract was seen on 6th day of treatment. It might be due to presence of phytoconstituents like L-dopa, polyphenols and flavonoids[18]. Due to presence of L-dopa in *Prosopis chilensis*, thus showing anti-depressant activity [19].

MPTP treated mice not only altered the behavioural response but also reduced the dopamine in the brain. These evidences suggest that these symptoms are similar to human Parkinsonism [18] and also our study in agreement with Ocimum sanctum extract contained precursors of dopamine and therefore exhibited antiparkinsonian activity [20].Other monoamines like Serotonin [21], Norepinephrine [22] were much less altered than dopamine in Parkinson disease. 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 parkinsons 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 [23]. Depression is a common symptom in patients with parkinsons 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 bean related to any manifestation of the disorder. Cerebrospinal fluid (CSF) content of the major major metabolite of serotonin, 5-hydroxyindoleacetic acid was lower in depressed than nondepressed parkinsonian's. The data suggest that the alterations in serotonin metabolism in Parkinson's disease identify a subgroup of patients who prone to depression [24].

The loss of dopamine and other amines was reversed well by alcoholic extract of PC because it might be due to cumulative effect of all components present in alcoholic extract of PC seed. The ethyl acetate and aqueous extract of PC had moderate effect it might be due to presence of specific component extracted in particular solvent. Pet ether extract of PC did not show significant effect on MPTP treated mice but only plant extract itself showed activity it could be due to presence of active components in low concentration.

Alcoholic and water extract of the plant might contain L-dopa and polyphenols in minute concentrations. Even though if L-dopa present in low concentration its efficiency was enhanced

by components and adjuvants present in alcoholic and water extract of PC seeds and these results were in agreement with [25].

Oxidative stress leads to organ damage, cell death. In the present study, we carried out reduced glutathione estimation and assessed lipid peroxidation in brain *in vivo.* Malondialdehyde (MDA) is an end product of lipid peroxidation [26]. In the present study, MDA level was increased in MPTP treated mice. This finding is similar to earlier reports [27]. Alcoholic and water extract of PC had good effect on decreased MDA level than other fractions, it could be due to presence of compounds like phenolic, L-dopa and flavonoids [28].
Reduced glutathione level was decreased in MPTP treated group [18], while their level was restored significantly with alcoholic, water and ethyl acetate extract of PC. Antioxidant activity was not only due to presence of flavonoids and polyphenols but also due to presence of alkaloids [29]. Preliminary phytochemical analysis of this plant showed the presence of flavonoids, phenolic compounds, amino acids, proteins and alkaloids. Hence, the antioxidant and free radicals scavenging activity of the plant might be due to presence of such compounds.

CONCLUSION

Prosopis chilensis improves dopamine loss in brain and also restored the antioxidants, among all fractions alcoholic and water fractions are more promising for further studies and may be useful for management of Parkinson's disease.

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