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RES ARTICLE

Neuroprotective effect of *Rhodiola rosea* Linn against MPTP induced cognitive impairment and oxidative stress

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ABSTRACT

Background: Ageing and age-related neurodegenerative changes including Parkinson's disease are characterized by an important role of reactive oxygen species. It is characterized by signs of major oxidative stress and mitochondrial damage in the pars compacta of substantia nigra. **Purpose:** Present study was designed to investigate whether *Rhodiola rosea* extract would prevent MPTP induced neurotoxicity in Male wistar rats. **Methods:** Male Wistar rats were divided into following five groups: Group I received vehicle (saline (10 ml/kg for 21 days) orally); Group II received *Rhodiola rosea* extract (250 mg/kg for 21 days) orally; Group III was treated with 20 mg/kg MPTP i.p. for 21 days; Group IV received 20 mg/kg MPTP, i.p. along with 100 mg/kg *Rhodiola rosea* orally for 21 days. Group V received 20 mg/kg MPTP i.p. along with 250 mg/kg *Rhodiola rosea* orally for 21 days. **Results:** MPTP induced rats showed behavioral alterations in elevated plus maze testing. Group III rats elicited significant increase in lipid hydroperoxide along with reduction in level of glutathione peroxidase, catalase, superoxide dismutase and total antioxidants. Histological evidence revealed that MPTP treated rats shown pathological changes like cellular inflammation and vascular degeneration in brain tissue. **Conclusion:** The oxidative stress and related biochemical alteration by MPTP were attenuated by *Rhodiola rosea* treatment. However, further studies may be necessary to elucidate the precise mechanism to support the clinical use of a plant source as antiparkinsonism drug.

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Introduction

Parkinson's disease is a common and debilitating age-associated human neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta and degenerating projecting nerve fibres in the striatum which leads to extrapyramidal function.[1] It is typified by four cardinal features such as bradykinesia, tremor, rigidity and postural instability.[2–4] Oxidative stress plays an important role in the pathology of parkinson's disease. It alters the mitochondrial function and increases the production of reactive oxygen species. Generation of high levels of reactive oxygen species and downregulation of antioxidant mechanisms results in cell death during ageing and age related neurodegenerative disorders including Parkinson's disease.[5] 1-methyl, 4 phenyl 1,2,3,6 tetrahydropyridine (MPTP) is a potent neurotoxin that induces PD in various experimental animals including monkeys, mice, cats, dogs, rats and goldfish.[6] MPTP is a highly lipophilic molecule crosses the blood brain barrier in a matter of seconds of systemic injection. It is taken up into astrocytes where it is metabolized to MPP^+ by monoamine oxidase-B. MPP^+ is taken up by dopamine neurons and causes a complex –I defect similar to that of Parkinson's disease.[7, 8] MPTP was used in the current study to mimic PD in rats which was then subjected to neuroprotective treatment. *Rhodiola rosea* belongs to the plant family crassulaceae (golden root) is widely distributed at high altitudes in the Arctic and mountainous regions throughout the Europe and Asia. It is a popular plant of traditional medical systems in Eastern Europe and Asia, with a reputation of stimulating the nervous system, decreasing depression, enhancing work performance, eliminating fatigue, and preventing high altitude sickness.[9] The roots and rhizomes of *Rhodiola rosea* are mainly responsible for pharmacological activity. The extracts of roots of *R. rosea* produce favorable changes in a variety of diverse physiological functions, including neurotransmitter levels, central nervous system activity and cardiovascular function. The phytochemistry of Rhodiola rosea root has revealed the presence of about 28 compounds classified into 6 distinct groups such as phenylpropanoids (rosavin, rosin, rosarin), phenyl ethanol derivatives (salidroside (rhodioloside), tyrosol), Flavanoids (rodiolin, rodionin, rodiosin, acetylrodalgin, tricin, Monoterpenes (rosiridol, rosaridin), Triterpenes (daucosterol, beta-sitosterol) Phenolic acids (chlorogenic and hydroxycinnamic, gallic acids).[10, 11] Extracts of the *R. rosea* root also contain powerful adaptogens which protected animals and humans against mental and physical stress, toxins, and cold.[12, 13] Salidroside, a phenyl propanoid derivative was reported to have adaptogenic properties.[14] p-tyrosol, phenolic acids such as gallic acid, caffeic acid, and chlorogenic acid), and flavonoids were reported to have antioxidant properties.[15, 16] With this evidence the neuroprotective action of Rhodiola rosea was investigated on MPTP rat model. Antioxidant compounds isolated from Rhodiola rosea are p-tyrosol, organic acids such as gallic acid, caffeic acid and chlorogenic acid and flavanoids such as catechins and proanthocyanidins. Significant free radical scavenging activity has been demonstrated from alcohol and water extracts of Rhodiola rosea is due to the presence of variety of antioxidant compounds.

Methods

Healthy male Wistar rats of 250-300 gm were used for the study. Animals were purchased from Govt Medical College, Thiruvananthapuram, Kerala, India and maintained under constant temperature with free access to animal food and water *ad libitum*. Animal ethical clearance was taken from the institute animal ethical committee and this study was conducted according to Animal Ethics Committee guidelines of our Institution.

Chemicals

MPTP-HCl was purchased from Sigma Aldrich, USA. Plant drug used for the study, *Rhodiola rosea* (RR) capsule extract was obtained from Biovea, Kensington, London. Commonly extract of *Rhodiola rosea* is available as capsules or tablets. All the chemicals used for the study was of analytical grade.

Experimental protocol

Rats used for the experiment were segregated into 5 groups with six animals in each group. Group I received normal saline 10 ml/kg orally (Control). Group II received 250mg/kg body weight of extracts orally for a period of 21 days (Drug control group). Group III received 20mg/kg body weight of MPTP administered intraperitoneally (PD model) for 21 days. Group IV received 20mg/kg body weight of MPTP along with 100mg/kg body weight of Rhodiola rosea extract (Low dose treatment group). Group V received 20mg/kg body weight of MPTP along with 250 mg/kg body weight of Rhodiola rosea extract (High dose treatment group).The drug Rhodiola rosea available in the form of Capsule. Each Capsule contains 300mg of Rhodiola rosea. For calculating 250mg/kg dose i.e. 250/1000 × 200= 50mg. For one rat 50mg of extract is needed. Here six rats are used for study. So 6 × 50=300mg needed for 6 rats.10 capsules were used for making solution. It means 10 capsules contain 3000mg powder. Then it was diluted to 30ml vehicle. 1ml of solution contains 100mg of extract. 0.5 ml of solution contains 50mg. For 100mg/kg dose i.e. 100/1000 × 200=20mg. For one rat 20 mg of extract is used. So 6 × 20 =120mg needed for 6 rats.5 capsules were used for preparation of drug solution. No of capsules i.e. 5 X weight of one capsule means 1500 mg for20 ml solution.1ml solution contains 75mg. 0.3 ml solution contains around 20mg. Behavioural studies was performed 2 weeks after MPTP treatment. Animals were sacrificed on 21st day of experimental protocol.

Behavioural studies

Elevated plus maze test

The elevated plus maze consisted of two opposite black open arms (50 × 10 cm), crossed with two closed walls of the same dimensions with 40 cm high walls. The arms were connected with a central square of dimensions 10 × 10 cm. The entire maze was elevated to a height of 50 cm from the floor. Acquisition of memory was tested on day 13 after MPTP administration. Animal was placed individually at one end of the open arm facing away from the central square. The time taken by the animal to move from the open arm to the closed arm was recorded as the initial transfer latency (ITL). Animal was allowed to explore the maze for 20 seconds after recording the ITL and then returned to the home cage. If the animal did not enter the enclosed arm within 90 seconds, it was guided on the back into one of the enclosed arm and the ITL was given as 90 seconds. Retention of memory was assessed by placing the rat in an open arm and the retention latency was noted on day 14 and day 21 of ITL and was termed as the first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively.[18]

Locomotor activity

Locomotor disability was measured using an Instrument called Actophotometer. The movement of animal cut off a beam of light falling on Photocell and count was recorded and displaced digitally. Each rat was placed individually in the actophotometer for 10 minutes and basal activity scores were recorded. Gross behaviour activity was observed on 14th and 21st day after MPTP injection. The animals were observed for a period of 10 minutes and values were expressed as counts/10 minutes.

Tissue collection

On completion of experimental period, animals are sacrificed by euthanasia under ketamine Anaesthesia. Brain tissues are excised immediately and immersed in ice cold saline. The tissues were homogenized in 0.01 M phosphate buffer solution of P^H7.4 using glass homogenizer. The homogenate was centrifuged at 12000 rpm for 20 minutes, 4°C to obtain the post mitochondrial supernatant (PMS) which was used for used for analyzing various biochemical parameters. The tissue homogenate was stored at -20°C until further use.

Histopathological analysis

Mid portion of the brain specimens obtained from all groups of animals were fixed in 10% formalin. The tissue sections were embedded in Paraffin wax and sectioned at 5-6 μm thickness and sections were stained with Haematoxylin and eosin method for photomicroscopic observation of the brain histopathological architecture.[19]

Biochemical analysis

From the homogenate samples glutathione peroxidase, catalase, superoxide dismutase were assayed using Elisa Kits.[20] The level of lipid peroxides were estimated by the method of Ohkawa *et al*.[21]

Statistics

The statistical analysis was carried using Graph pad Instat software of version 3. Hypothesis testing methods included one way analysis of variance followed by Newman Keul's Multiple range test. P values less than 0.01 was considered to statistical significance. All these results were expressed as mean ± SE for six animals in each group.

Results

Table I shows *Rhodiola rosea* improved on behavioural alterations in MPTP treated rats; The mean initial transfer latencies (ITL) on day 13 for each rat was relatively stable and showed no significant variation among different groups. Mean retention transfer latencies (1st RTL and 2nd RTL) to enter closed arm on day 14 and 21 were shorter as compared to ITL on day 13 of each group, respectively. MPTP injected rats did not show any change in the mean retention time transfer latencies on day 14 and 21 as compared to the pre training latency on day 13, demonstrating that MPTP induced marked memory impairment. Chronic Administration of RR (100 mg & 250 mg/kg) beginning prior to MPTP injection significantly decreased mean retention latencies on day 14 & 21 following MPTP injection ($p<0.01$).

Table 2 shows antioxidant status; LPO levels in the brain tissue homogenate were significantly elevated ($P<0.01$) and reduction in the activities of SOD, catalase, glutathione peroxidase and total antioxidants in MPTP treated animals (Group III) relative to controls. RR (250 mg/kg) administration of MPTP treated animals tend to bring the SOD, CAT, GPx and TA levels close to normal values. No significant were found in rats treated with RR alone.

Table 3 shows effect on locomotor activity. The mean scores of locomotor activity for each rat were more relatively stable and showed no significant variation among different groups. The mean scores in normal control, perse control and MPTP treated rats remain unchanged. Further, both the dose of RR

Table 1: Assessment of Cognitive performance in MPTP treated rats

Groups	Treatment	Mean transfer latency(sec)		
		ITL	1st RTL	2nd RTL
Group I	Normal control (10 ml/kg. N. Saline)	60.10 ± 2.08	21.6 ± 1.90	22.0 ± 1.60
Group II	Perse control (250 mg/kg of R. R)	64.6 ± 2.16	19.4 ± 1.82	18.0 ± 1.72
Group III	Toxic control (20 mg/kg of MPTP)	68.77 ± 2.62	82.30 ± 3.40*a	80.6 ± 3.16*a
Group IV	Treatment I/Low dose treatment (100 mg/kg R. R + MPTP)	65.6 ± 2.12	48.60 ± 2.52*b	43.4 ± 2.06*b
Group V	Treatment II/High dose treatment group (250 mg/kg RR + MPTP (20 mg/kg)	66.6 ± 2.30	38.4 ± 2.42**b	34.2 ± 1.92**b

Statistical significance was represented as P<0.01. a** Group III compared with Group I and Group II. b* Group IV compared with Group III. b** Group V compared with Group III.

Table 2: Antioxidant status in homogenate of brain tissues.

Groups	Lipidhydroperoxide LPO (nmol/mg of protein)	Total antioxidants TA (mM/mg of protein)	Glutathione Peroxidase GP_x (nmol/min/mg of protein)	Catalase CAT (μM/mg of protein)	Superoxide dismutase (units/mg of protein)
Group I	3.03 ± 0.10	3.27 ± 0.30	23.8 ± 1.38	48.60 ± 3.32	35.73 ± 3.19
Group II	2.51 ± 0.30	3.10 ± 0.16	26.18 ± 1.95	53.80 ± 4.23	33.02 ± 1.05
Group III	4.24 ± 0.25a**	1.57 ± 0.15a**	12.65 ± 1.83a**	31.26 ± 2.84a**	9.82 ± 0.58a**
Group IV	2.13 ± 0.13b*	2.38 ± 0.15b*	20.46 ± 2.6b*	44.78 ± 2.39b*	19.17 ± 1.47b*
Group V	2.52 ± 0.15b**	2.77 ± 0.13b**	22.01 ± 1.56b**	47.49 ± 2.24b**	23.03 ± 1.97b**

Statistical significance was represented as P<0.01. a** Group III compared with Group I. b* Group IV compared with Group III, Group V compared with Group III.

Table 3: Table demonstrating locomotor activity of different treatment groups

GROUPS	TREATMENT	Locomotor activity (score) in 10 min±SEM on	
		14th day	21st day
GROUP I	Normal Control	218 ± 12.85	220.00 ± 11.45
GROUP II	Perse Control	207.5 ± 10.20	212.40 ± 10.40
GROUP III	Toxic Control	222.4 ± 13.4	232.6 ± 12.40
GROUP IV	Low dose treatment group	201.5 ± 9.6	228.4 ± 11.0
GROUP V	High dose treatment group	230.4 ± 12.4	230.5 ± 12.6

(100 mg&250 mg/kg) did not cause any significant activations in the locomotor activity as compared to MPTP treated rats on day 14&21.

Histopathological studies of midbrain (Figure 1) (1. Histopathological study of midbrain portion is considered because Substantia nigra is a part of the midbrain which contains a large number of dopamine producing neurons. MPTP is a site specific toxin which damages the dopamine producing neurons in substantia nigra) viewed under light microscope in control and experimental animals. Haematoxylin/eosin staining of paraffin sections (100 XH&E). A: Section of brain from rats treated with normal saline

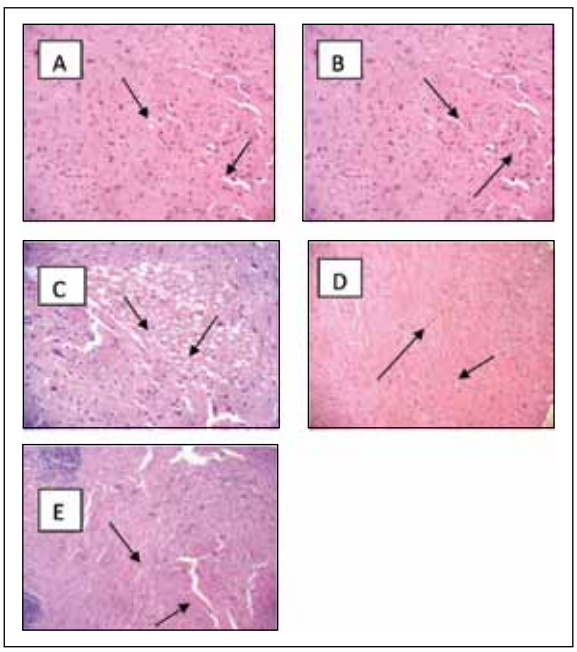

Fig. 1: A portion of the midbrain where substantia nigra is located stained with Haematoxylin and eosin viewed under light microscope (100 X H&E). **A:** Rats treated with Normal Saline for 21 days **B:** Rats treated with 250 mg/kg body weight of Rhodiola rosea for 21 days (perse control). **C:** Rats treated with 20 mg/kg MPTP for 21 days **D& E:** Rats treated with MPTP for 21 days followed by RR treatments.

for 21 days showing normal architecture. B: Section of brain from rat treated with 250 mg/kg body weight of *Rhodiola rosea* for 21 days (perse control) showing normal architecture. C: section of brain from rats treated with MPTP for 21 days showing pathological changes like cellular inflammation, vascular degeneration and cytoplasmic vacuolation. D & E: Section of brain from rats treated with MPTP for 21 days followed by RR treatments showing marked reduction of degeneration and vacuolation.

Discussion

There is a growing evidence that oxidative stress and mitochondrial respiratory failure with attendant decrease in energy output are implicated in neuronal death in PD.[22] MPTP is believed to induce selective toxicity at central dopaminergic neurons via the end products of its oxidation, 1-methyl-4-phenyl pyridinium (MPP^+) which inhibits oxidative metabolism at complex I of the mitochondrial respiratory chain by its specific inhibition of NADH-Ubiquinone oxidoreductase.[23] Although the mechanism by which it induces selective dopaminergic cell death has not been fully elucidated, it was described that MPTP acts as an inhibitor of Complex-I of mitochondrial respiratory pathway and produces a decrease in tissue ATP content and increases ROS formation.[24] Certain brain regions like striatum and hippocampus are highly enriched with non-heme iron, which is catalytically involved in the production of ROS.[25] Exposure to MPTP might have lead to the peroxidation of membrane lipids eventually leading to the loss of membrane integrity and finally lead to cell death in these brain regions. The differences in the level of LPO products observed in various brain regions may be attributed to the differences in their iron content and diverse metabolism, which influence the generation of ROS. Malonised dopaminergic neurons have been reported to be more susceptible to neurodegeneration in PD and MPTP toxicity.[26]

During oxidative stress in the neuronal cells there is an increase in intracellular calcium levels in the brain.[27] This increased intracellular calcium levels can induce the irreversible conversion of Xanthine dehydrogenase (XDH) to XO, which inturn catalyzes the oxidation of Xanthine to provide a source of oxygen. Inaddition, auto-oxidation of dopamine in brain could also serve as a source of superoxide anion.[28] These mechanism could be the main reasons for the increased levels of XO and reduction in activity of SOD leading to an overload of oxygen radicals and repression of antioxidant enzymes with MPTP exposure.

The present study evidenced that oxidative stress plays an important role in neurodegeneration caused by MPTP. A significant rise in LPO levels observed in MPTP challenged rats when compared to control rats. RR administration reduced LPO significantly in MPTP challenged rats. The reduction in LPO levels may be due to the electron and H^+ donating capacity of polyphenols present in RR, which is attributed to the termination of LPO chain reaction based on their reducing power. Several studies have shown that flavanoids interact with cell membranes, improving their stability, thereby protecting them from LPO.[29] The concentration of GP_x, CAT, SOD and the TA were found to be decreased significantly in the brain tissue of MPTP treated rats as compared to the control rats. RR treatment increases the level of Th GP_x, CAT, SOD and the TA in MPTP challenged rats. The antioxidant property of RR may possibly be attributed to the nitrigenomic phenolic compounds present which are also effective hydrogen donors, which makes them good antioxidants.[30] MPTP when given intraperitoneally resulted in significant memory impairment in elevated plus maze which was attenuated by chronic RR treatment for 21 days.

In conclusion, the present study implies that *Rhodiola rosea* enriched with bioflavanoids, polyphenols and triterpenes may be particularly useful xenobiotics detoxifying agents as it could decrease lipid peroxidation and enhance brain antioxidants and significantly prevent the brain from neurotoxic effects of MPTP. Therefore, *R. rosea* could offer a useful support to the Parkinsonism therapy by acting as a neuroprotective antioxidant and thus prevent the neuronal damage in brain regions associated with Parkinsonism. Thus RR extract enriched with bioflavanoids polyphenols and triterpenes may be considered as a powerful neuroprotective agent for membrane molecular medicine. Further studies may be necessary to elucidate the exact molecular mechanisms of actions of the various constituents in RRE against MPTP induced excitotoxicity.

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