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PROANTHOCYANIDIN RICH FRACTION OF *M. NAGI* BARK (PMN) ATTENUATES RESERPINE-INDUCED IMPAIRMENT OF COGNITION AND LOCOMOTION IN EXPERIMENTAL ANIMALS

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Abstract: Reserpine induced orofacial dyskinesia is a well know experimental animal model of Parkinsonism. Generation of free radicals in presence of Reserpine is responsible foroxidative stress induced neurological damage. Reserpine impairs memory and also affects locomotor activity. Anti-oxidant compounds may help in reversal of such neuroleptic induced impairment of cognition and locomotion. In this study, proanthocynidin rich fraction of M. nagi(PMN) possessing antioxidant activity was evaluated for protective effect against reserpine induced cognitive and locomotor impairment. PMN significantly increased number of squares traversed and number of self and supported rearing and transfer latency as compared to reserpine treated group. Reserpine also increased supported rearing compared to self-rearing.

Keywords: Cognition; Locomotor activity; Myrica nagi; Reserpine

1. Introduction:

Plants having free radical scavenging activity are useful in the treatment of neurodegenerative diseases. There is a general agreement that flavonoids act as scavengers of reactive oxygen species [1]. The in-vitro antioxidant properties of proanthocynidins from Myrica nagi (PMN) bark could be attributed to the presence of flavonoid phytoconstituent in it. Bark contains a variety of flavonoids [2,3,4] and steroids, reducing sugars, tannins, and glycosides, saponins and volatile oils [5].

So, in the view of above literature, the present study was planned to study the effect of proanthocynidins rich fraction from Myrica nagi bark on reserpine-induced impairment of cognition and locomotion in Wistar rats.

2. Materials And Methods:

2.1 Animals:

Male Wistar rats (150-200 g) and Swiss albino mice (18-22 g) were obtained from Bharat Serum and Vaccine Ltd., Thane. All the experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics Committee (IAEC) of M.G.V.'s Pharmacy College, Panchavati, Nashik (Protocol number: MGV/PC/XXX/01/15). Ethical guidelines were strictly followed during all the experiments.

2.2 Drugs and chemicals:

Reserpine (Sigma-Aldrich, USA) was administered subcutaneously. Vitamin E was purchased from Merck, Mumbai. All drug solutions were freshly prepared in saline before each experiment; Proanthocynidins isolated from Myrica nagi bark was dissolved in distilled water and administered orally.

2.3 Isolation of Proanthocynidins:

Dried bark of Myrica nagi Thub was purchased from local market of Nashik and authenticated by Dr. (Mrs) A. G. Bhaskarwar from Ayurved Mahavidyalaya, Panchavati, Nashik, India. The bark was used for preparation of proanthocyanidin-rich fraction [6].

2.4 Acute oral toxicity studies:

Acute oral toxicity of PMN was performed in Swiss albino mice using OECD (Organization of Economic Co-Operation Development) guidelines 423. During the first 4 h after the drug administration, animals were continuously observed for gross behavioral changes and then observation was continued for 24 h and 72 h in regular intervals for 14 days.

Since, none of the animals died at 2000 mg/kg (oral) even after fourteen days, doses of 100, 200 and 400 mg/kg were selected for further studies.

2.5 Impairment of cognition and locomotion using reserpine:

Rats were divided in 6 groups, (n=5), received vehicle, reserpine (1 mg/kg, s.c.) in 0.1% acetic acid, PMN (100, 200 and 400 mg/kg, p.o), and Vitamin E (10 mg/kg, p.o.) one hour after reserpine. Reserpine was administered to rats for 5 days on every alternate day [7, 8]. Effect on locomotor activity and cognitive performance was evaluated on day 7, 14 and 21.

2.5.1 Locomotor activity using Open Field Apparatus:

The Locomotor activity was monitored using Open field apparatus [9]. The rats were observed in a square open field arena (68 × 68 × 45 cm3). Measurements were made in the dark, in a ventilated, sound-attenuating room on day 7, 14 and 21 for 5 min. The locomotor activity was expressed in terms of number of squares traversed, number of self and assisted rearing.

2.5.2 Cognitive performance using Elevated Plus Maze:

Animals were placed individually at the end of either of the open arms of the EPM facing away from the central platform. The time taken by each animal to move from end of open arm to either of the closed arms was recorded. This duration of time is called as Transfer latency (TL). If the animal does not enter into any of the enclosed arms within 90 sec, it shall be gently pushed into any of the enclosed arms and the TL was assigned as 90 sec. Later the animal was allowed to explore

the plus maze for 5 min after the measurement of TL and sent back to home cage. TL was then noted on 7th, 14th and 21st day. TL measured on 1st day serves as a parameter for acquisition (learning) while TL on 7th, 14th and 21st day indicates retention (memory) [10].

2.6 Statistical analysis:

The mean ± SEM values were calculated for each group. One-way ANOVA followed by Dunnett's multiple comparison tests were used for statistical analysis. Values of $p<0.05$, $p< 0.01$, and $p< 0.001$ were considered statistically significant.

3. Results:

3.1 Effect of Proanthocynidins from M. nagi bark (PMN) on Locomotor activity in reserpine treated rats:

3.1.1 Number of squares traveled:

In open field apparatus, Reserpine treatment significantly decreased number of squares traveled by rats. Treatment with PMN (100, 200 and 400 mg/kg, p.o for 21 day) and Vitamin E (10 mg/kg p.o for 21 day) significantly increased number of squares traveled as compared to reserpine treated group, (Fig .1).

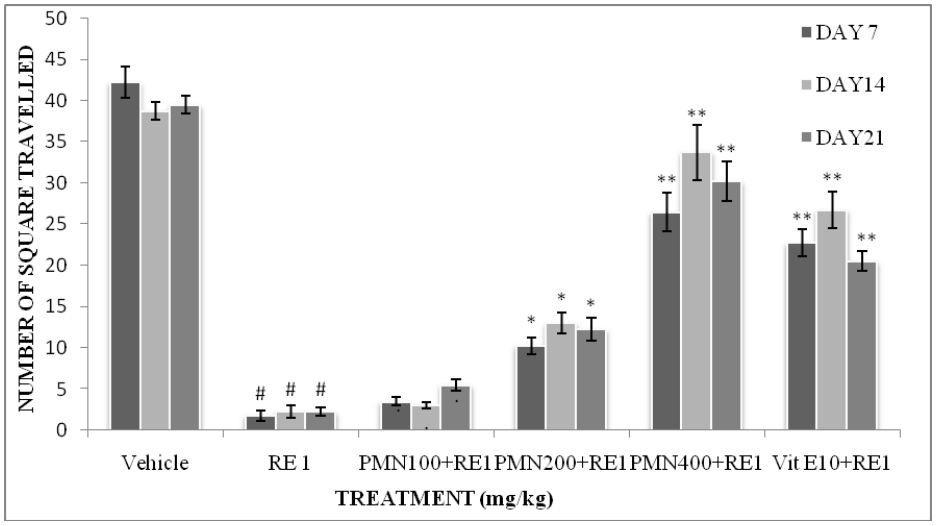

Fig. 1: Effect of Proanthocynidins from Myrica nagi bark (PMN) on number of squares traveled.

All data expressed as mean ± SEM at n = 5, # $P<0.001$ compared to vehicle treated group.
*$P<0.01$, **$P<0.001$, compared with reserpine treated group, (One-way ANOVA followed by Dunnett's test).

3.1.2 Self-rearing in reserpine treated rats:

In open field apparatus, Reserpine treatment significantly ($P<0.001$) decreased number of self rearing in rats. Treatment with PMN (100, 200 and 400 mg/kg, p.o for 21 day) and Vitamin E (10 mg/kg p.o for 21 day) significantly increased number of self rearing as compared to reserpine treated group, (Fig. 2).

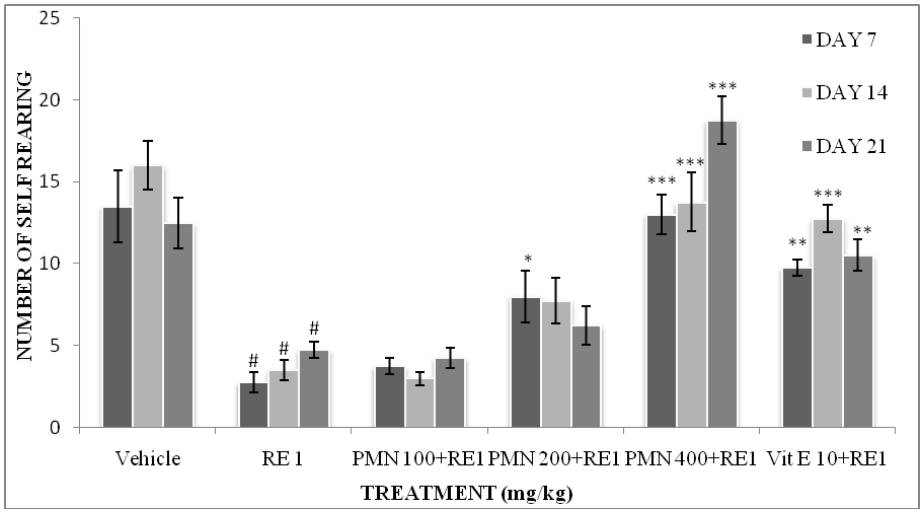

Fig. 2: Effect of Proanthocynidins from Myrica nagi bark (PMN) on self-rearing in reserpine treated rat.

All data expressed as mean ± SEM at n = 5, # P<0.001 compared to vehicle treated group.
*P<0.05, **P<0.01, ***P<0.001, compared with reserpine treated group, (One-way ANOVA followed by Dunnett's test).

3.1.3 Supported rearing in reserpine treated rats:

In open field apparatus, Reserpine treatment significantly decreased number of supported rearing in rats. Treatment with PMN (100, 200 and 400 mg/kg, p.o for 21 day) and Vitamin E (10 mg/kg p.o for 21 day) significantly increased number of supported rearing as compared to reserpine treated group (Fig. 3).

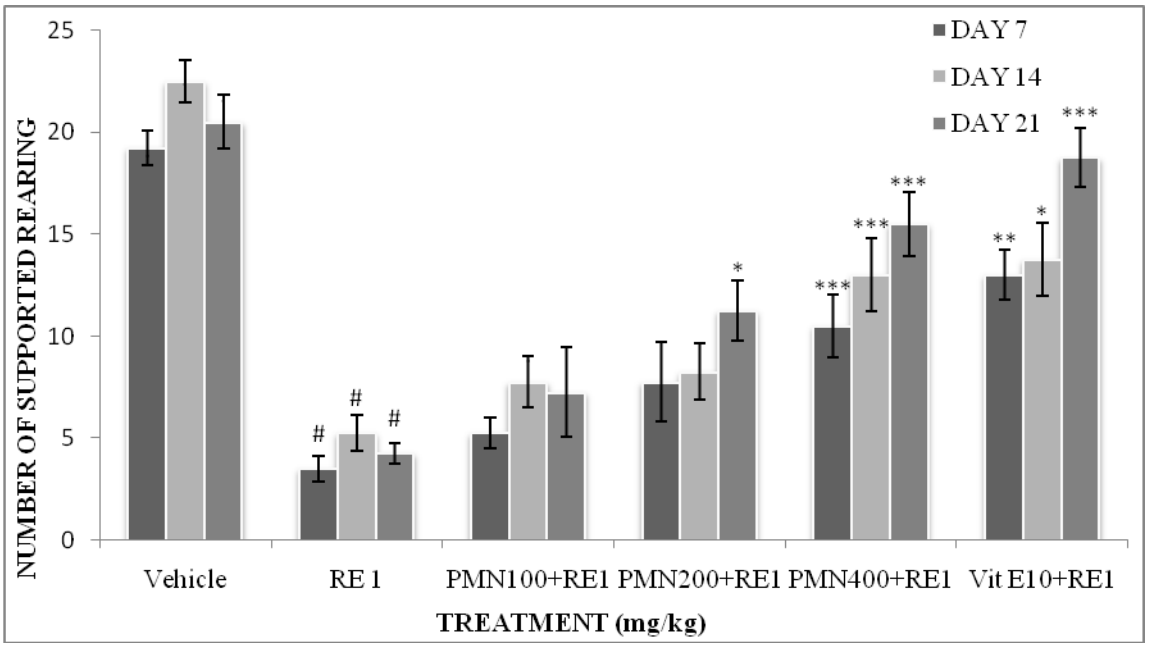

Fig. 3: Effect of Proanthocynidins from Myrica nagi bark (PMN) on supported rearing in reserpine treated rat.

All data expressed as mean ± SEM at n = 5, # P<0.001 compared to vehicle treated group.

*P<0.05, **P<0.01, ***P<0.001, compared with reserpine treated group, (One-way ANOVA followed by Dunnett's test).

3.2 Effect of Proanthocynidins from M. nagi bark (PMN) on cognition in reserpine treated rats:

In elevated plus maze, Reserpine treatment significantly decreased transfer latency in rats. Treatment with PMN (100, 200 and 400 mg/kg, p.o for 21 day) and Vitamin E (10 mg/kg p.o for 21 day) significantly increased transfer latency as compared to reserpine treated group, (Fig. 4).

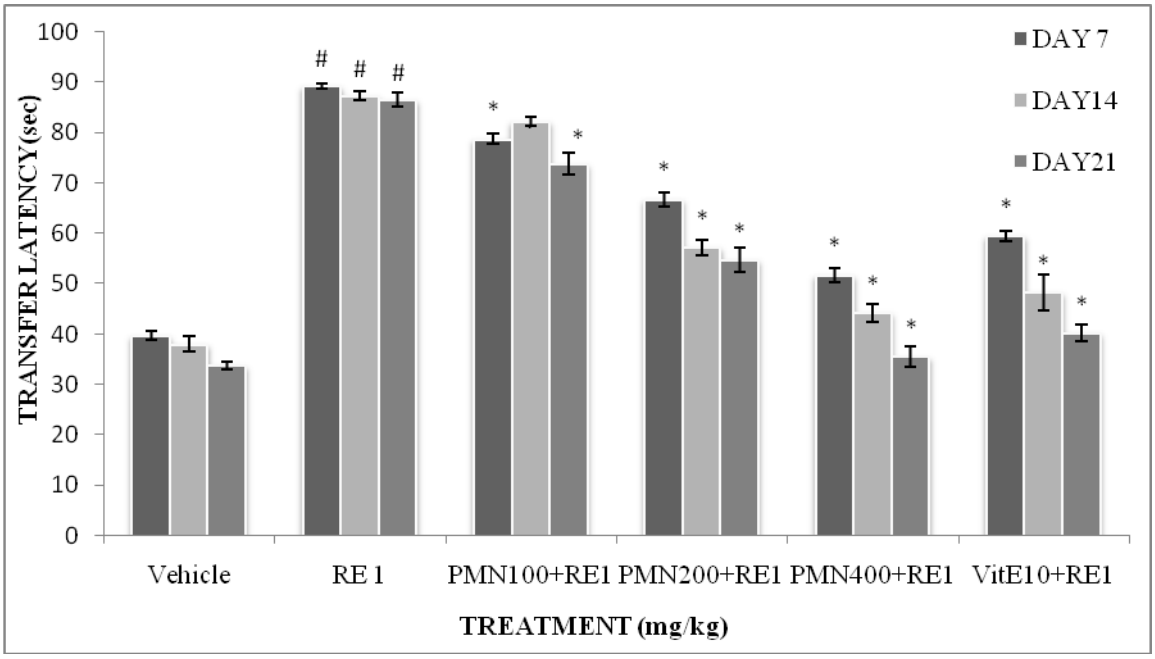

Fig. 4: Effect of Proanthocynidins from Myrica nagi bark (PMN) on cognition in reserpine treated rats

All data expressed as mean ± SEM at n = 5, # P<0.001 compared to vehicle treated group.
*P<0.001 compared with reserpine treated group, (One-way ANOVA followed by Dunnett's test).

4. Discussion:

The brain and nervous system are particularly prone to free radical damage since the membrane lipids are very rich in polyunsaturated fatty acids and certain areas of brain are very rich in iron, which favor generation of free radicals [11, 12, 13]. The metabolism of catecholamines, such as dopamine and norepinephrine are probably associated with free radical formation and conditions associated with increased catecholamine metabolism may increase the free radical burden. As free radicals are potentially toxic, they are usually inactivated or scavenged by antioxidants before they can impose damage to lipids, proteins or nucleic acids.

Tardive dyskinesia (TD) induced by an antipsychotic compound Reserpine [14] provides an animal model of TD that has been associated with free radical generation and oxidative stress [15].

Neuroleptics also act by blocking dopamine receptors [16] Such blockade results in increased dopamine turnover [17], which in turn could conceivably lead to an increased production of hydrogen peroxide, resulting in oxidative stress [18,19].

Reserpine blocks monoamine storage, leading to a depletion of dopamine, norepinephrine, and serotonin. Considerable evidence indicates that low doses of apomorphine can decrease dopaminergic transmission through relatively selective actions on dopamine autoreceptors [20,21]. Thus, it is possible that depletion of dopamine with reserpine treatment decreased dopamine release produced by injection of 0.1 mg/kg apomorphine, served to produce substantial decreases in dopamine release that led to dramatic increases in vacuous jaw movements.

In present study the phytochemical screening of proanthocynidins isolated from M. nagi bark showed presence of flavonoids, phenolic compounds and tannins [22]. These compounds are known to possess potent antioxidant activity. Flavonoids act as scavengers of reactive oxygen species [1]. The antioxidant activity of M. nagi extract is confirmed by the in-vitro antioxidant methods. The antioxidant properties of PMN could be attributed to the presence of flavonoids in it [23]. Dopamine and noradrenaline are involved in the control of motor activity.

In open field apparatus, reserpine treatment decreases number of squares traveled and number of self and supported rearing in rats. Treatment with PMN and Vitamin E significantly increased number of squares traveled and number of self and supported rearing as compared to reserpine treated group. Reserpine also increased supported rearing compared to self-rearing. Reserpine treatment decreases Transfer latency in rats. Treatment with PMN and Vitamin E significantly increased Transfer latency compared to reserpine treated animals.

Conclusion:

Thus, it may be concluded that proanthocyanidin-rich fraction of M. nagi bark (PMN) is useful in preventing reserpine-induced amnesia and damage to locomotor activity.

Conflict of interest:

Authors report no conflict of interest regarding authorship and publication of this article.

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