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Research Article

PROTECTIVE EFFECT OF PROANTHOCYNIDINS FROM *M. NAGI* BARK (PMN) ON RESERPINE-INDUCED OROFACIAL DYSKINESIA IN EXPERIMENTAL ANIMALS

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ABSTRACT

Reserpine-induced neurodegeneration and free radical damage causing orofacial dyskinesia is an animal model for Parkinsonism. Aim of work was to assess neuroprotective and *in-vivo* free radical scavenging activity of proanthocynidins isolated from bark of *Myrica nagi* (PMN). In the existing study, vacuous chewing movements (VCM) and orofacial bursts (OB) in Wistar rats were induced by subcutaneous administration of reserpine (1 $mgkg^{-1}$) on day 1, 3 and 5. Effect of PMN on VCM, OB and *in-vivo* antioxidant defence enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH), extent of lipid peroxidation (LPO) and dopamine was studied in forebrain region. PMN significantly reversed reserpine induced VCM, OD. PMN treatment significantly elevated levels of SOD, CAT, GSH and inhibited LPO. PMN also restored levels of dopamine in forebrain region indicating potential role of PMN is reversal of reserpine induced neurodegeneration and Parkinson's disease.

Keywords: *Myrica nagi*, Orofacial dyskinesia, Reserpine, Tongue protrusion, Vacuous chewing movements.

1. INTRODUCTION

The neurodegenerative hypothesis proposed in the mid-1980s indicates that persistent TD may be associated with or triggered somewhere in the basal ganglia by damage to neurons. In exchange, these neurons affect the inhibitory effects of GABAergic neurons on the dopaminergic output neurons located in the nigrostriatal tract, potentially contributing to an excess or hyper-functional state of dopamine in the basal ganglia. Building on that theory, it is proposed that presynaptic catecholaminergic fibers and other neurotransmitter systems could be involved in the impairment affecting function of neuronal membrane and, might lead to cell death if remains unchecked [1]. Neuroleptic treatment causing neuronal changes were secondary to free radical generation after metabolism and turnover of dopamine. At some stage, a threshold may be reached for antioxidant defense mechanism involving catalase, superoxidase [2].

Oxidative stress may be causative factor in reserpine-induced OD [3]. Oxidative stress can cause neurodegenerative disorders like Alzheimer's disease, Parkinson's disease. PD is due to the participation of free radicals in the process. Flavonoids serve as scavengers of reactive oxygen species [4]. Flavonoid phytoconstituent present in the *Myrica nagi* (PMN) bark could be responsible for *in-vitro* antioxidant properties. Bark contains a variety of flavonoids [5, 6], reducing sugars, steroids, glycosides, tannins, saponins and volatile oils [7].

So, in the view of above literature, the existing work was planned to study the effect of proanthocynidins from *Myrica nagi* bark on reserpine-induced orofacial dyskinesia in Wistar rats.

2. MATERIAL AND METHODS

2.1. Animals

Swiss albino mice (18-22 g) and male Wistar rats (150-200 g) were procured from Bharat Serum and Vaccine Ltd., Thane. At an ambient temperature of 25±1°C, the animals were housed in groups of five. IAEC of the MGVs Pharmacy College, Panchavati, Nasik approved the protocol of this study (Protocol number: MGV/PC/XXX/01/15), under the provisions of the CPCSEA, Government of India. During all the experiments, tests ethical directives were strictly followed.

2.2. Drugs and chemicals

Reserpine (Sigma-Aldrich, USA) was administered subcutaneously, TBA (Sigma, USA), DPPH (Sigma, USA), Trichloro-acetic acid, Phosphate buffer, pH 7.4 (Qualigens Fine Chemicals, Mumbai), ethylene-diamine-tetra-acetic acid (EDTA) were used for this study. Before each experiment, drug solutions were prepared freshly using saline. Distilled water was used as vehicle for Proanthocynidins isolated from *Myrica nagi* bark and extract was 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. Proanthocynidins from *M. nagi* bark (yield: 1.12% w/w) were extracted by method of Harborne. Chemical tests for phyto-constituents present in the PMN bark such as flavonoids and tannins were carried out [8].

2.4. Acute toxicity studies

Swiss albino mice were used for determination of acute oral toxicity of PMN using OECD-423 guidelines [9]. Since, none of the animals died at 2000 $mgkg^{-1}$ (oral) even after fourteen days, doses selected for further study were 100, 200 and 400 $mgkg^{-1}$.

2.5. Reserpine-induced orofacial dyskinesia

Rats were alienated in 6 groups (n=5), received vehicle, reserpine (1 $mgkg^{-1}$, s.c.) in 0.1% acetic acid, PMN (100, 200 and 400 $mgkg^{-1}$, p.o), and Vitamin E (10 $mgkg^{-1}$, p.o.) one hour after reserpine. Orofacial dyskinesia was induced in rats with reserpine administration for 5 days on every alternate day [10, 11]. After administration of reserpine, rats were placed individually in a small transparent box (22 x 22 x 22 cm^3). Rats were observed for 5 min and vacuous chewing movements (VCM) and orofacial bursts (OB) were scored.

2.5.1. Body weight

Change in body weight after treatment with PMN extracts in reserpine-treated rats was observed on day 7, 14, and 21.

2.5.2. Vacuous chewing movement and Orofacial bursts

VCM and OB were scored for a period of 5 min by immediately placing rats individually in a small box (22x22x22cm^3) after reserpine treatment.VCM and OB numbers were scored for a period of 5 min. Effect of PMN on VCM of rats after treatment with reserpine was observed on day 7, 14 and 21.

2.5.3. Catalepsy

The catalepsy period was assessed for 3 hours at an interval of 30 min using the Bar test [12] on day 7, 14 and 21. A bar raised to 8cm height was used to put forepaws of rats. For each animal, time required to remove forepaw from the bar was noted.

2.5.4. Biochemical Analysis

2.5.4.1. Determination of in vivo antioxidant status

On the 22nd day of reserpine therapy, shortly after behavioral tests, decapitation method was used for scarification of the animals. For dissection of forebrain, brain was isolated, and was rinsed using isotonic saline and weighed. A 10 percent (w/v) tissue homogenate was prepared using phosphate buffer, the post-nuclear fraction was used for catalase assay and further centrifuged at 12000 rev/minute for other enzyme assays.

2.5.4.2. Assessment of Superoxide dismutase(SOD)

The SOD assay is based on ability of SOD to suppress spontaneous oxidation of adrenochrome by adrenaline [13, 14]. SOD delays rather than inhibits the reaction and the diminished formation of adrenochrome due to the scavenging of superoxide anions. Absorbance was noted at 480 nm. The findings were represented as SOD in Unit mg^{-1}.

2.5.4.3. Assessment of Catalase (CAT)

Catalase assay is based on the degradation of hydrogen peroxide by CAT [15]. The absorbance was registered for 1 min every 10 seconds at 240 nm. The findings were expressed as wet tissue of catalase in Umg^{-1}.

2.5.4.4. Assessment of reduced glutathione (GSH)

Reduced glutathione was measured using Ellman's method [16]. In a spectrophotometer, yellow color of NTB^{2-}ion is quantified by measuring the absorbance at 412nm. The findings were expressed as units of GSH $mg^{-1.}$

2.5.4.5. Assessment of extent of lipid peroxidation (LPO)

Thiobarbituric acid reacts with malonaldehyde to form Thiobarbituric acid reactive substances (TBARS) chemical reaction. Method of Niehaus et al. [17] was used for measurement of extent of lipid peroxidation, as

demonstrated by the formation of TBARS. Absorbance was measured at 535nm. The outcomes were represented as wet tissue LPO nmol/mg.

2.5.4.6. Assessment of dopamine levels in brain

Immediately after measurement of catalepsy on 21 day of experiment, rats from each group were sacrificed by decapitation. The brains were separated; dissected forebrains were rinsed with isotonic saline, weighed and dopamine content was assessed using filter fluorimeter-(Model-CL-53, ELICO Pvt Ltd) [18].

2.6. Statistical analysis

For each group, the mean ± SEM values were determined. A one-way ANOVA was used for statistical analysis. Dunnett's multiple comparison tests was used after ANOVA. Values of $p< 0.05$, $p< 0.01$, and $p< 0.001$ were considered statistically significant.

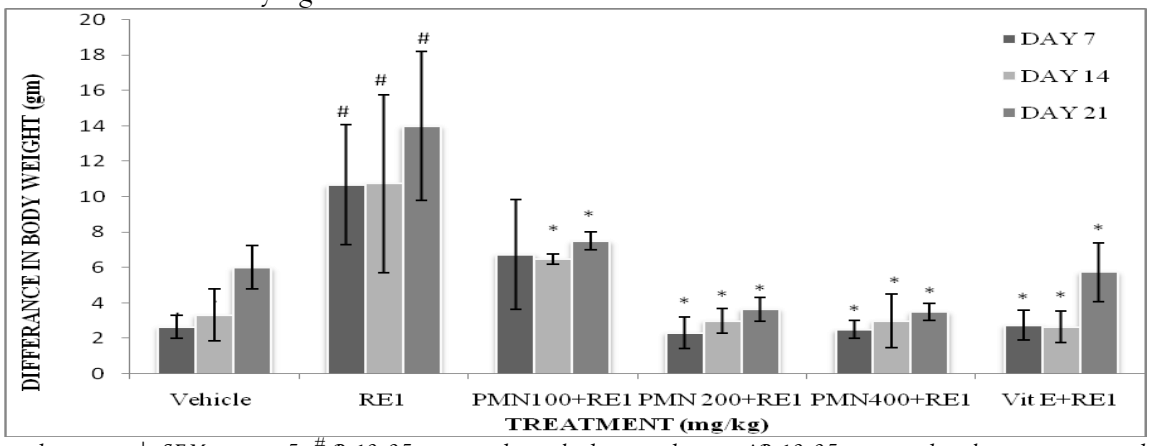
3. RESULTS

3.1. Body weight

Reserpine treated animals exhibited significantly ($P < 0.05$) increased body weight when compared to vehicle treated animals. Treatment with PMN and Vitamin E for 21 day in reserpine treated animals significantly ($P < 0.05$) inhibited increase in the body weight compared to the reserpine treated group (Fig.1).

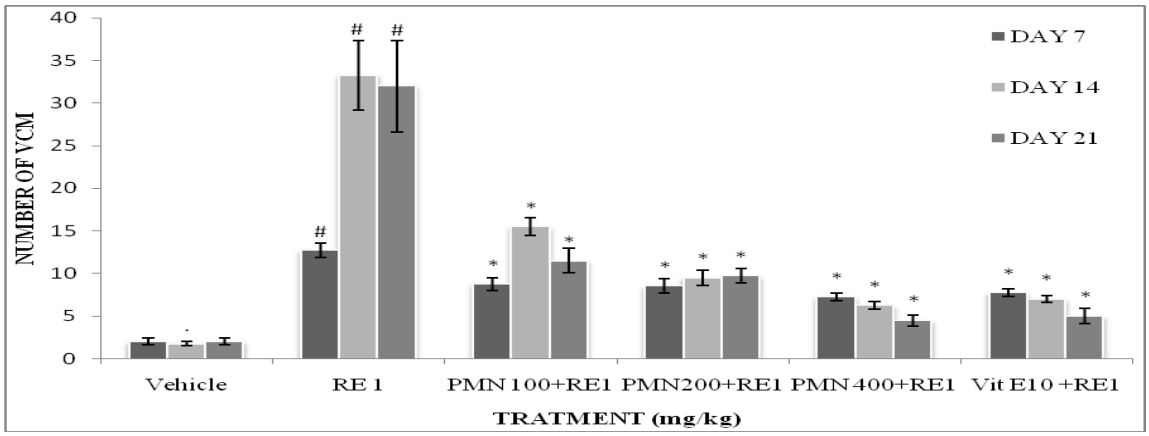
3.2. Vacuous chewing movements (VCM)

Reserpine treated group exhibited significantly ($P<0.001$) increased number of VCM in rats compared to vehicle treated group. Treatment with PMN and Vitamin E for 21 days significantly reduced VCM compared to the reserpine treated group ($P<0.001$), (Fig. 2).



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

Fig. 1: Effect of Proanthocynidins from *Myrica nagi* bark (PMN) on body weight in reserpine treated rat



*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).*

Fig. 2: Effect of Proanthocynidins from *Myrica nagi* bark (PMN) on reserpine- induced vacuous chewing movements in rats

3.3. Orofacial Bursts (OB)

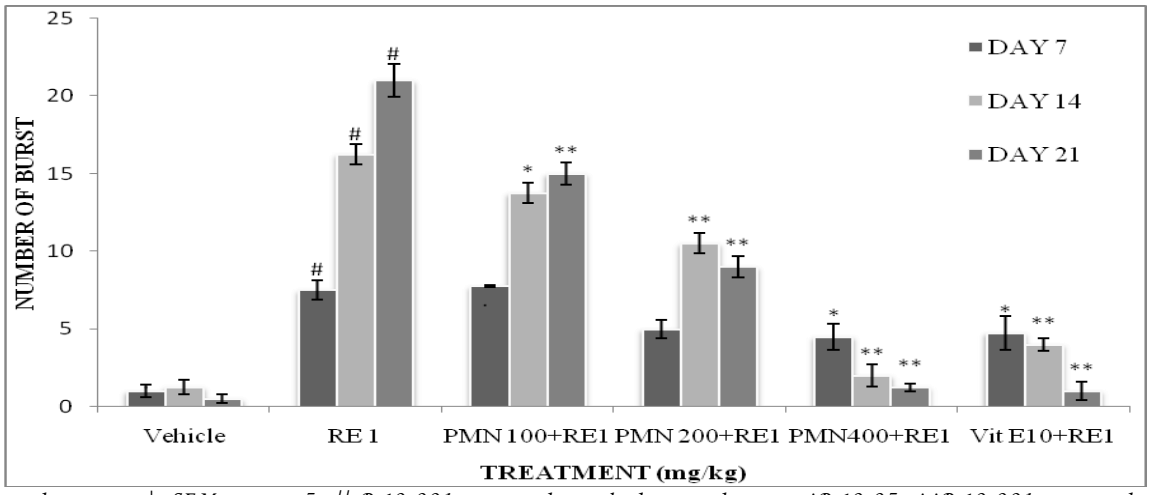
Reserpine treated group exhibited significantly ($P< 0.001$) increased number of OB in rats compared to vehicle treated group. Treatment with PMN and Vitamin E for 21 days significantly reduced number of OB compared to the reserpine treated group ($P<0.05$, $P<0.001$) (Fig. 3).

3.4. Catalepsy

Reserpine produced catalepsy (Sharma et al, 2011), which remained for 3 hours. Maximum duration of catalepsy was at 180 min after reserpine. A significant reduction in catalepsy was observed in the PMN and Vitamin E treated group on day 7, 14 and 21 when compared with the reserpine (Figs.4-6).

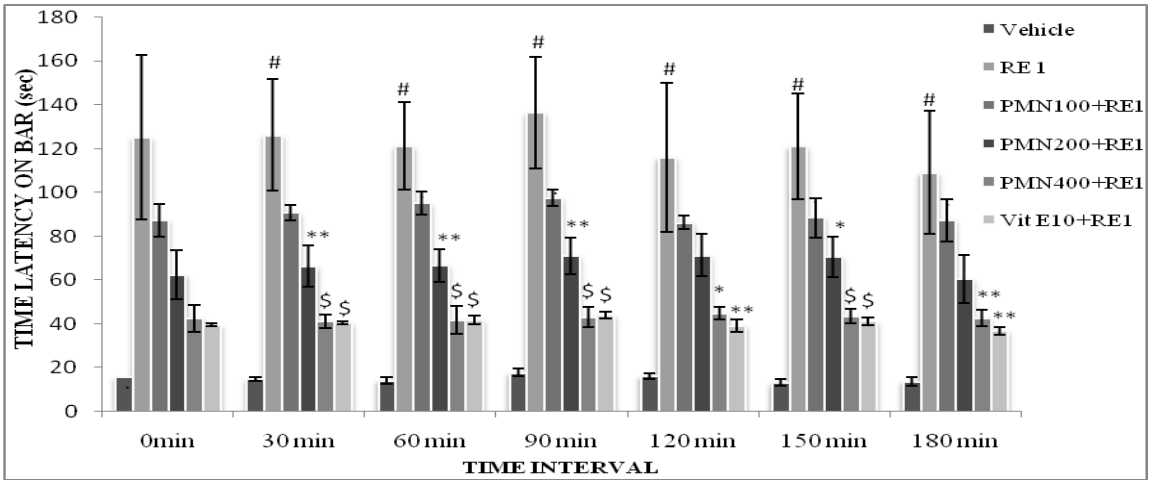
3.5. Anti-oxidant enzyme levels in the forebrain

Reserpine treated rats exhibited significantly reduced forebrain SOD levels. PMN and Vitamin E treatment for 21 days significantly inverted the reserpine-induced decrease in SOD (Fig.7), CAT (Fig.8) and GSH (Fig. 9) levels as compared to reserpine treated rats.



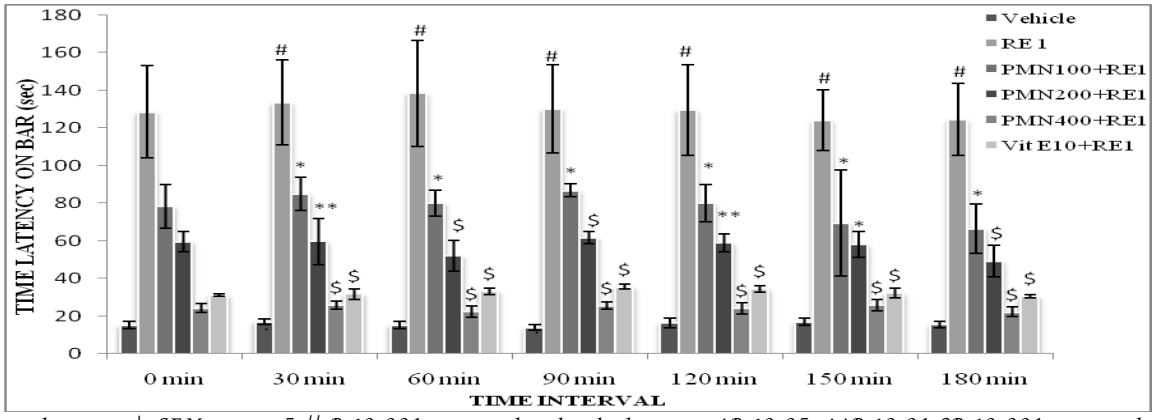
*All data expressed as mean ± SEM at n = 5, # P<0.001 compared to vehicle treated group. *P<0.05, **P<0.001 compared with reserpine treated group, (One-way ANOVA followed by Dunnett'stest).*

Fig. 3: Effect of Proanthocynidins from *Myrica nagi* bark (PMN) on reserpine- induced Orofacial Bursts in rats



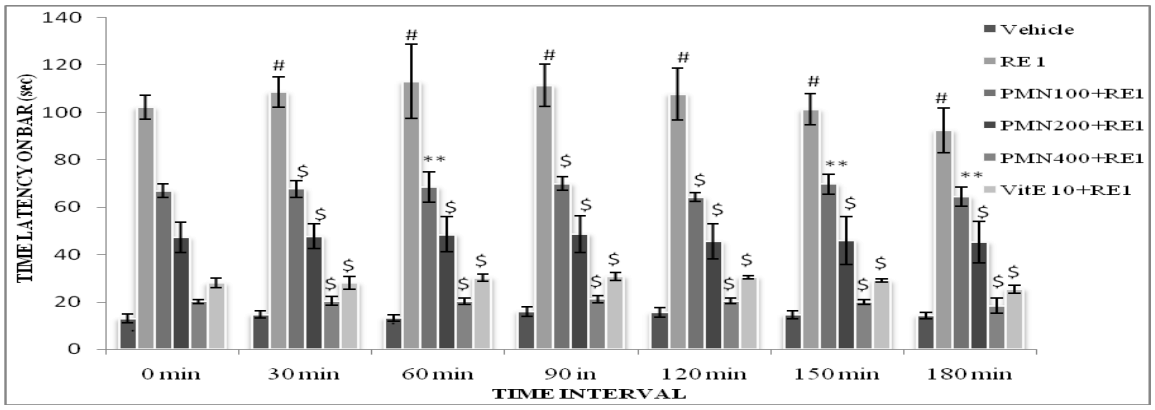
*All data expressed asmean ± SEM at n = 5,# P<0.001 compared with vehicle group.*P<0.05, **P<0.01, $P<0.001, compared with reserpine treated group, (One-way ANOVA followed by Dunnett's test).*

Fig. 4: Effect of Proanthocynidins from *M. nagi* bark (PMN) on reserpine -induced catalepsy in rats on 7th day



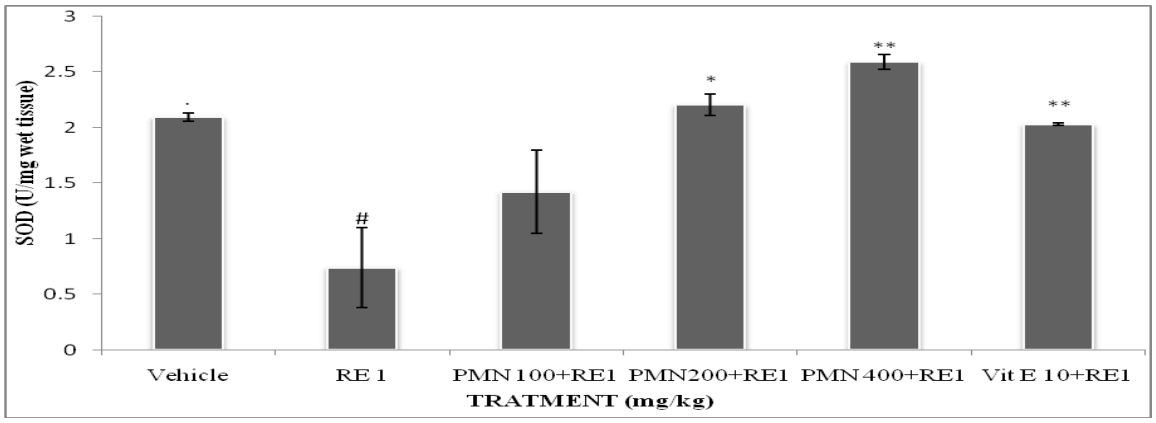
*All data expressed asmean ± SEM at n = 5,# P<0.001 compared with vehicle group. *P<0.05, **P<0.01 $P<0.001 compared with reserpine treated group, (One-way ANOVA followed by Dunnett's test).*

Fig. 5: Effect of Proanthocynidins from *M. nagi* bark (PMN) on reserpine -induced catalepsy in rats on 14th day.



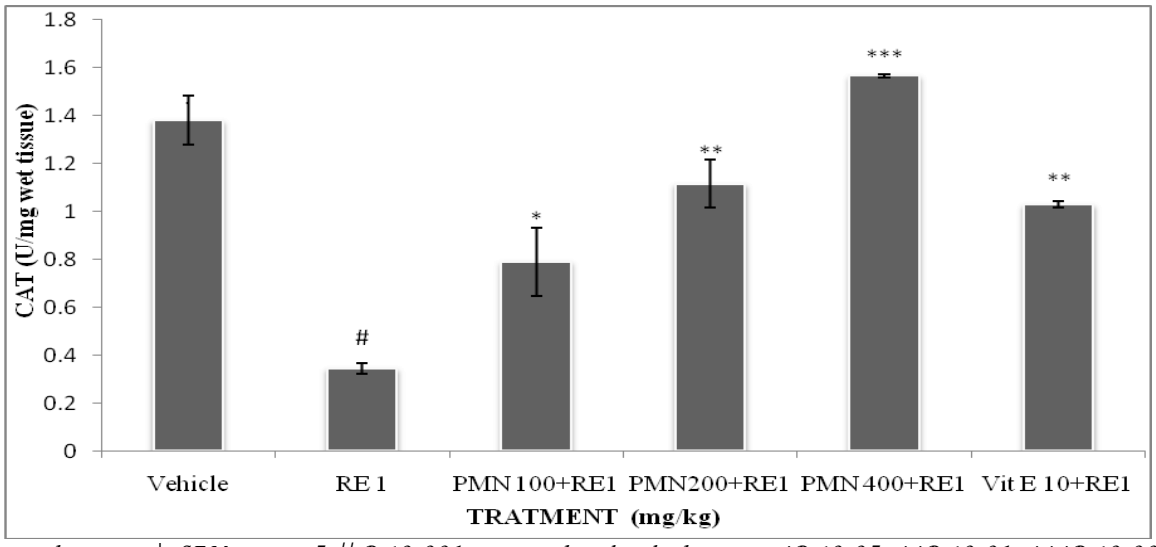
*All data expressed asmean ± SEM at n = 5,# P<0.001 compared with vehicle group. **P<0.01, $P<0.001, compared with reserpine treated group, (One-way ANOVA followed by Dunnett's test).*

Fig. 6: Effect of Proanthocynidins from *M. nagi* bark (PMN) on reserpine -induced catalepsy in rats on 21st day



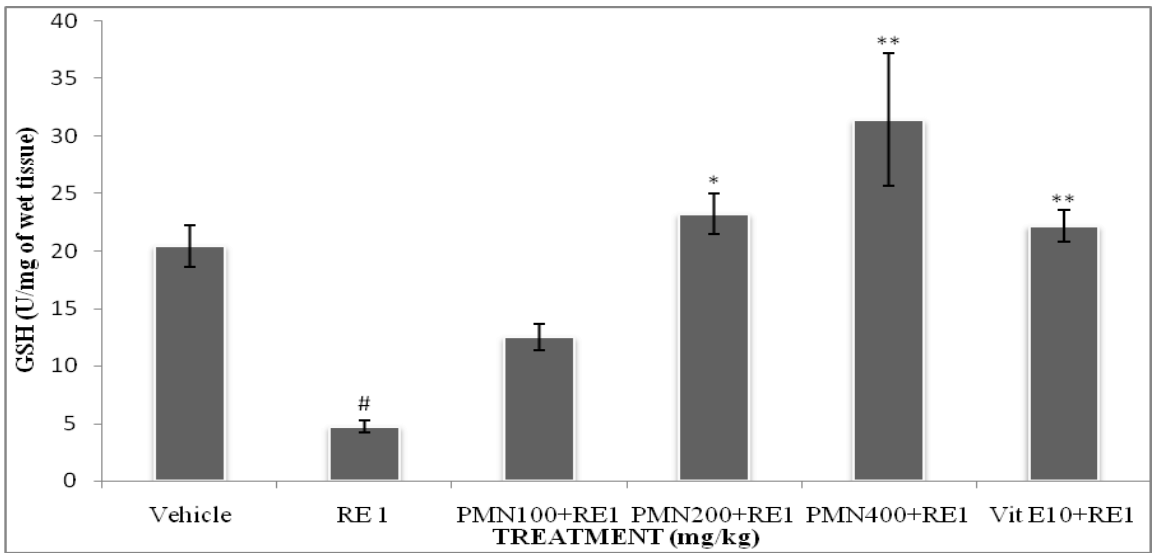
*All data expressed as mean ± SEM at n = 5, # P<0.01 compared with vehicle group.*P<0.05, **P<0.01 compared with reserpine treated group, (One-way ANOVA followed by Dunnett's test).*

Fig. 7: Effect of Proanthocynidins from *M. nagi* bark (PMN) on SOD levels in the forebrain in reserpine treated rats



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

Fig. 8: Effect of Proanthocynidins from *M. nagi* bark (PMN) on CAT levels in the forebrain in reserpine treated rats



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

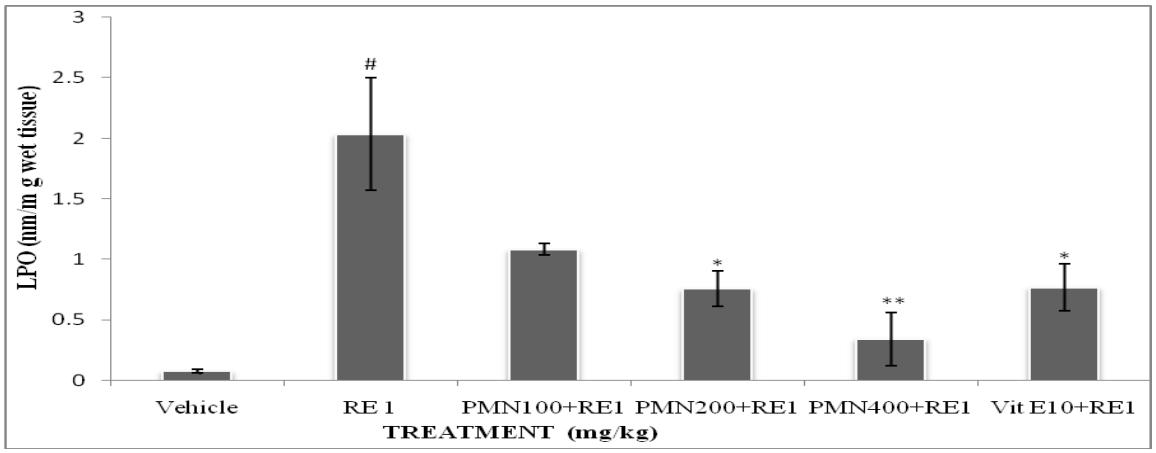
Fig. 9: Effect of Proanthocynidins from *M. nagi* bark (PMN) on GSH levels in the forebrain in reserpine treated rats

3.6. Extent of lipid peroxidation (LPO)

Reserpine treatment significantly brought lipid peroxidation shown by significant increase in MDA levels in forebrain compared to control group. Significant reversal in LPO was observed compared to reserpine treated group when PMN and vitamin E for 21 day were administered along with reserpine (fig.10).

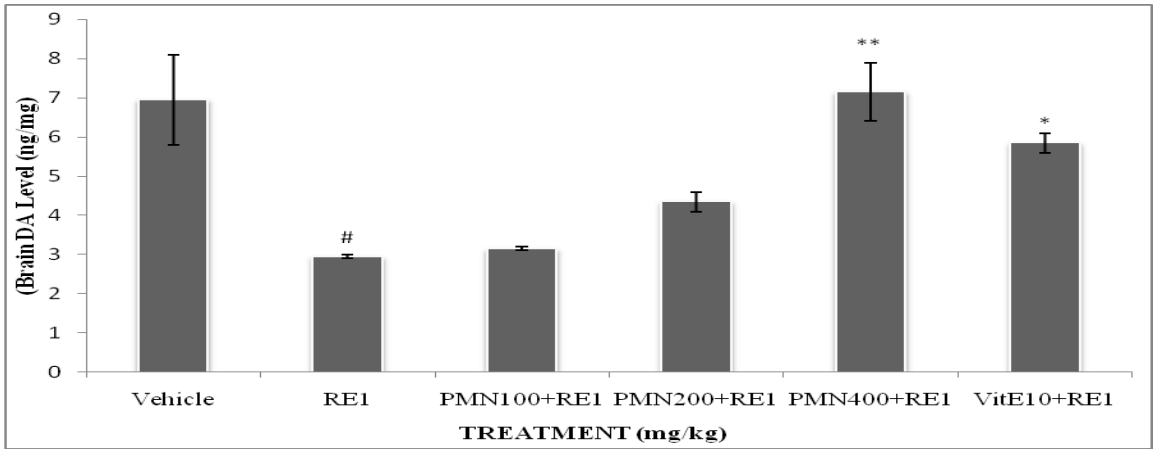
3.7. Forebrain Dopamine levels

Dopamine levels in forebrain homogenates were significantly reduced in reserpine treated rats. Significant reversal in the forebrain levels of dopamine compared to reserpine treated rats were observed after treatment with PMN and vitamin E for 21 day (Fig.11).



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

Fig. 10: Effect of Proanthocynidins from *M. nagi* bark (PMN) on extent of lipid peroxidation (LPO) in the forebrain in reserpine treated rats.



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

Fig. 11: Effect of Proanthocynidins from *M. nagi* bark (PMN) on the forebrain Dopamine levels in reserpine treated rats

4. DISCUSSION

As polyunsaturated fatty acids are present abundantly in membrane lipids, the brain and nervous system are more prone to free radical damage. Certain areas of brain contain high iron that favors free radical generation [19-21]. Free radicals' development may be allied with metabolism of catecholamines including norepinephrine and dopamine, resulting in increased burden of free radicals. Free radicals show potential toxicity and antioxidants get inactivated or scavenged until they impair proteins, lipids and nucleic acids.

As an antipsychotic agent, reserpine was used and was correlated with the development of Tardive dyskinesia (TD) [22]. Based on these correlations, oral dyskinesia induced by reserpine offers an animal TD model associated with generation of oxidative stress and free radicals [23-25]. The capacity of antipsychotics to induce substantial gain in weight [26,27]. Appetite stimulation can also occur by antagonism of the hypothalamus-located receptors D2, M1, H_1 [28] and 5-HT_{2c} [29, 30]. Treatment with PMN for 21 days in reserpine treated animals significantly inhibited-weight gain. Significantly higher levels of TBARS in striatum were observed in rats with VCM indicating production of lipid peroxidation and free radicals [31].

The human body has a complex protection mechanism for antioxidants that includes SOD, CAT and GSH antioxidant enzymes. These enzymes block free radical

chain reaction initiation [32]. Diminished activity of antioxidant defense enzymes viz. SOD and CAT is associated with repeated use of neuroleptics [31, 33].

Neuroleptics also act by blockade of dopamine receptors [34]. Increasing dopamine turnover [35] leading to increased hydrogen peroxide development and oxidative stress [31, 36]. Reserpine inhibits the storage of monoamine, which leads to dopamine, norepinephrine, and serotonin depletion. Important evidence suggests that low apomorphine doses can reduce dopaminergic transmission through relatively selective behavior on auto-receptors of dopamine [37, 38]. Therefore, dopamine depletion with reserpine treatment can decrease the release of dopamine produced by 0.1 $mgkg^{-1}$apomorphine injection, resulting in significant decreases in the release of dopamine, leading to drastic increases in vacuous jaw movements.

Useful behavioral signs of central cholinomimetic drug actions may be oral behaviors such as VCM and OB. Observing mouth movements can provide data on the potency, efficacy and time course of cholinomimetics in vivo. In addition, it presents a new model of behavior that may enable researchers to further explore cholinergic mechanisms of the brain.

The association between cholinergic-related oral behaviors and those seen during stimulant-induced stereotypes is important to speculate on. Rupniak et al. [39] proposed that an acute dystonic reaction, possibly linked to the activation of Parkinsonian symptoms, represents the oral behavior caused by neuroleptics.

Antiparkinsonian medications are associated with decrease in reserpine-induced jaw movements may be linked to impact of dopamine D1 and D2 agonists on in vivo activity of striatal acetylcholine. It is known that cholinergic stimulation induces Parkinsonian symptoms, and evidence suggests that dopamine antagonism or depletion can improve the release of striatal acetylcholine [40]. Free radical scavenger and an endogenous antioxidant, Vitamin E, is one of the most promising recent TD therapies [41].

In this study, the phytochemical screening of proanthocynidins isolated from *M. nagi* bark indicated presence of flavonoids, phenolic compounds and tannins [42]. These compounds are known to possess potent antioxidant activity. Reactive oxygen species are scavenged by flavonoids [4]. The antioxidant activity of *M. nagi* extract is confirmed by the *in-vitro* antioxidant methods and it could be due to the flavonoids present in it.

The regulation of motor activity includes dopamine and noradrenaline. The growth of orofacial dyskinesia in rats requires free radicals. Significant inhibition in reserpine-induced VCM and OB in rats was observed after oral treatment of PM Nand Vitamin Efor21 days. Reserpine treatment indicates a time-dependent catalepsy. Compared to reserpine-treated animals, catalepsy was substantially inhibited by PMN and vitamin E. Reserpine-treated animals have shown a rise in lipid peroxidation levels and decreased levels of GSH and defensive antioxidant enzymes such as SOD and CAT in the current research, indicating a potential generation of free radicals. Reserpine-induced oral dyskinesia has also been shown in previous research to be closely associated with oxidative stress. Reserpine administration also decreased the Dopamine level in brain. Treatment with PMN and Vitamin E attenuated these increased levels of Dopamine in brain. Dopamine content of brain increases with increasing dose of PMN. The results are in association with studies by Shimmyoet al [43]. They studied effect of myricetin isolated from *M. nagi* bark on brain dopamine levels. Myricetin restored dopamine levels in animal models of Parkinsonism.

5. CONCLUSION

It can be inferred, therefore, that proanthocynidins from *M. nagi* bark are beneficial in order to avoid reserpine-induced orofacial dyskinesia and Parkinson's disease.

6. ACKNOWLEDGEMENT

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Conflict of interest

Authors report no conflict of interests.

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