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Research Article

Piper nigrum Mitigates Reserpine-Induced Orofacial Dyskinesia in Wistar Rats

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Abstract

Piper nigrum is well known for antioxidant activity. Neurodegenerative disorders including Parkinsonism involve oxidative stress as major cause. Existing study was conducted to explore effect of ethanolic extract of P. nigrum (PN) fruits on orofacial dyskinesia induced by reserpine in Wistar rats. Body weight, behavioural and biochemical parameters were evaluated. PN significantly reversed parameters of reserpine induced orofacial dyskinesia including vacuous chewing movements, tongue protrusions and orofacial bursts. PN reversed cognitive impairment induced by reserpine assessed using elevated plus maze and locomotion assessed using open field apparatus. PN also reversed levels of anti-oxidant enzymesviz superoxide dismutase, catalase, glutathione reductase and extent of lipid peroxidation and exhibited protective effect against oxidative damage induced by reserpine.

Keywords: *Neurodegeneration, Oxidative Stress, Piper nigrum, Reserpine*

Introduction

According to neurodegenerative hypothesis proposed in the mid-1980s for Tardive Dyskinesia (TD), persistent TD may be associated with, or induced by, neuronal damage somewhere in the basal ganglia. Cadet and Lahr suggests that damage, which can affect neuronal function and, if left untreated result in cell death involving presynaptic catecholaminergic fibres and other neurotransmitter systems [1]. Neuroleptic agents causing these secondary neuronal changes due to free radical generation from increased dopamine metabolism. Increased dopamine conversion caused by neuroleptics causes overproduction of these free radicals which can be harmful. In some cases, the antioxidant immune system comprising superoxide dismutase (SOD), catalase, and glutathione enzyme complex, peroxidase, reductase and antioxidants such as vitamin E may be disrupted after reaching a threshold [2].

Reserpine-induced OD is associated with oxidative stress [3]. Parkinson's disease involves generation of free radicals. Herbal medicines with the free radical radicals scavenging actions are helpful in the management of neurodegenerative diseases. Natural antioxidants in plants are gaining popularity as a result of the worldwide major shift toward using natural ingredients in food and cosmetics [4,5]. Because to its pungency, fragrant odour, and flavour, black pepper is renowned as "The King of Spices" or "Black Gold" in many parts of the world [6]. *Pipernigrum*L. (Family: Piperaceae) shows significant antioxidant activity [7].

Existing study was aimed to explore the benefit of *P. nigrum* using various parameters of orofacial dyskinesia in experimental animals.

Materials and Methods

Animals

Male Wistar rats (150 - 200 gm) were procured from Bharat Serum and Vaccine Ltd., Thane and housed under normal light and temperature conditions. All procedures used in this study are approved by the IAEC, M.G.V.'s Pharmacy College, Nashik (Protocol Number: MGV-PC- XXVI-01/2011 - 12). The guidelines of CPCSEA were stringently adhered to during the entire trial.

Drugs

Reserpine obtained from Sigma-Aldrich, USA and Vitamin E by Merck, Mumbai were used in this study.

Plant material

P. nigrum Fruits obtained from local market, Nasik were authenticated by Dr. P.G. Diwakar, Jt. Director, BSI, Pune, where a voucher specimen (ARBAPI1) has been deposited. Dried fruit powder (1 kg) of *P.nigrum* was extracted with 2 L Ethanol (95%) in Soxhlet apparatus for 15 days (32 cycles). The solution was filtered and concentrated with constant stirring to get extract (PN) (Yield: 2.5% ww^{-1}).

Phytochemical analysis of PN

Phytochemical analysis were performed to identify presence of phytochemical constituents in the extract [8]. Phytochemical analysis revealed presence of flavonoids, phenolic compounds, tannins, carbohydrates and saponins.

Reserpine's effect on orofacial dyskinesia (OD)

The rats were separated into five groups (n = 5) and given the following treatments: vehicle, subcutaneous reserpine (1 $mgkg^{-1}$), oral PN (100 and 300 $mgkg^{-1}$), or oral Vitamin E (10 $mgkg^{-1}$) 1 h after reserpine. Reserpine every other day for 5 days was given to rats for development of OD [9,10]. Following reserpine injection, rats were put separately in a tiny Plexiglas cage (22 x 22 x 22 cm^3). The effect of extracts on rat body weight following reserpine administration was noticed. For a 5-minute period, the number of VCM, OB, TP, and grooming was counted.

Locomotor activity

Open field equipment placed in the dark, sound-attenuating room was used to track locomotor activity [11,12]. On days 7, 14, and 21, the rats were watched for 5 minutes in a square open field (68x68x45 cm^3). Number of self and assisted rearing and squares traversed were used to measure locomotor activity.

Cognitive performance

Elevated plus maze (EPM) raised to a height of 50 cm and 2 open arms facing opposite each other was used. Individual rats were positioned at end of each of the EPM's open arms facing away from the central platform. Transfer latency (TL) was measured. After measurement of TL, rat was permitted to explore EPM for 5 minutes before being returned to its home cage. On the 7^{th}, 14^{th}, and 21^{st}days, TL was recorded. The first day's TL represents acquisition (learning), whereas the seventh, fourteenth, and twenty-first days' TL reflect retention (memory) [12,13].

In vivo antioxidant status

After behavioural assessments, animals were sacrificed. Forebrain was isolated and weighed after being washed with isotonic saline. Phosphate buffer (0.1 M and pH 7.4) was used for preparing 10% w v-1 homogenate. The post nuclear fraction was generated by centrifuging the homogenate at 4°C at 1000 rev/minute for 20 minutes for the catalase assay, and at 12000 rev/minute for 60 minutes for the other enzyme assays.

Superoxide dismutase (SOD) activity estimation

Inhibition of conversion of adrenaline to adrenochrome was expressed as change in optical density every minute at 480 nm against reagent blank. Results were reported in SOD activity units per milligrams of wet tissue [14,15].

Catalase activity estimation

Disappearance of hydrogen peroxide was expressed as catalase activity units per milligrams of wet tissue after measurement of absorbance at 240 nm every 10 seconds for 1 minute [16].

Reduced glutathione estimation

Reaction of thiols with DTNB gives 2-nitro-5-thiobenzoate (NTB^-), which ionizes to yellow coloured NTB^{2-} dianion. Results reported as GSH activity units per milligrams wet tissue after measurement of absorbance of visible light at 412 nm [17].

Lipid peroxidative indices (LPO) estimation

Lipid peroxidation is expressed in terms of Thoi barbituric acid reactive substances (TBARS) formed after reaction of TBA with malonaldehyde. Results were expressed as activity of LPO in terms of nmol/mg wet tissue after measuring absorbance at 535 nm [18].

Statistical analysis

Values expressed as mean ± SEM (n = 5). One-way ANOVA followed by Dunnett's multiple comparison tests were used for statistical analysis. Statistical analysis done using GraphPad Instate (V.3.01) and GraphPad Prism (V.5.01).$^{\#}p < 0.05$ compared with vehicle group.$^{\#\#}p < 0.01$ compared with vehicle group. *p < 0.05 compared with reserpine treated group. **p < 0.01 compared with reserpine treated group was considered statistically significant.

Results

Body weight: Reserpine treated animals (1 mg/kg,s.c.on day 1, 3, 5) exhibited significantly elevated body weight compared to vehicle treatment. Significant reversal of increased body weight was observed after *P. nigrum* treatment compared to reserpine treated animals (Figure 1).

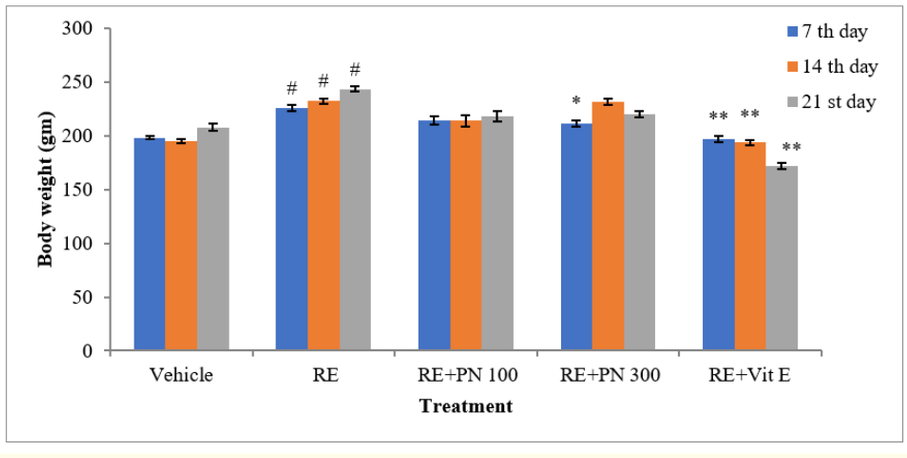

Figure 1: *Effect of P. nigrum on body weight in reserpine treated rat.*

Vacuous chewing movements (VCM)

Reserpine treatment group exhibited significant increase inVCM compared to vehicle treatment. *P. nigrum* significantly reduced VCMs (Figure 2).

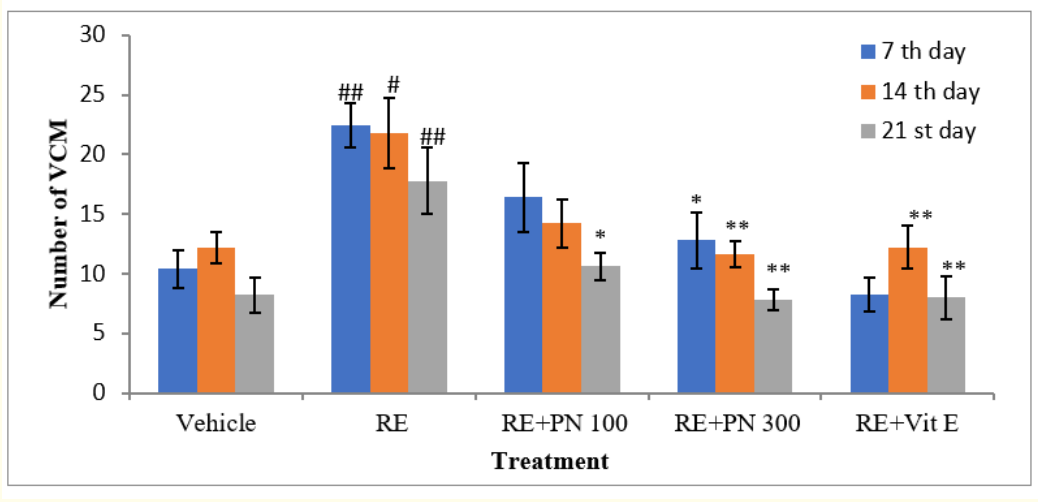

Figure 2: *Effect of P. nigrum on reserpine-induced VCMs in rats.*

Orofacial bursts (OB)

Reserpine treatment group increases number of orofacial bursts in rats compared with control. Pretreatment with *P. nigrum* fruit ethanolic extractsignificantly reduced number of orofacial bursts (Figure 3).

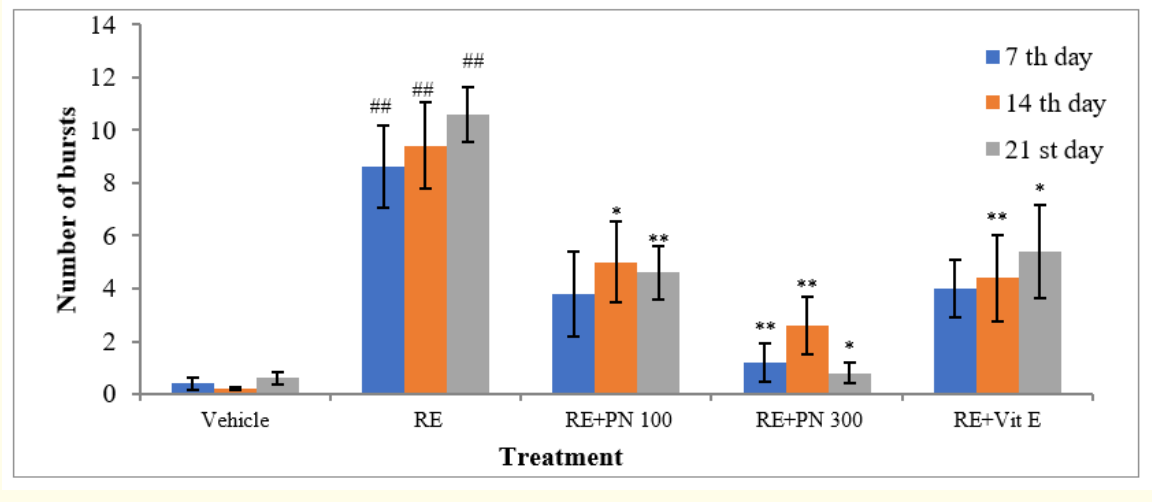

Figure 3: *Effect of P. nigrum on reserpine-induced OBsin rats.*

Tongue protrusions (TP)

Reserpine treatment group increases number of TP in rats. *P. nigrum* significantly reduced number of tongue protrusion (Figure 4).

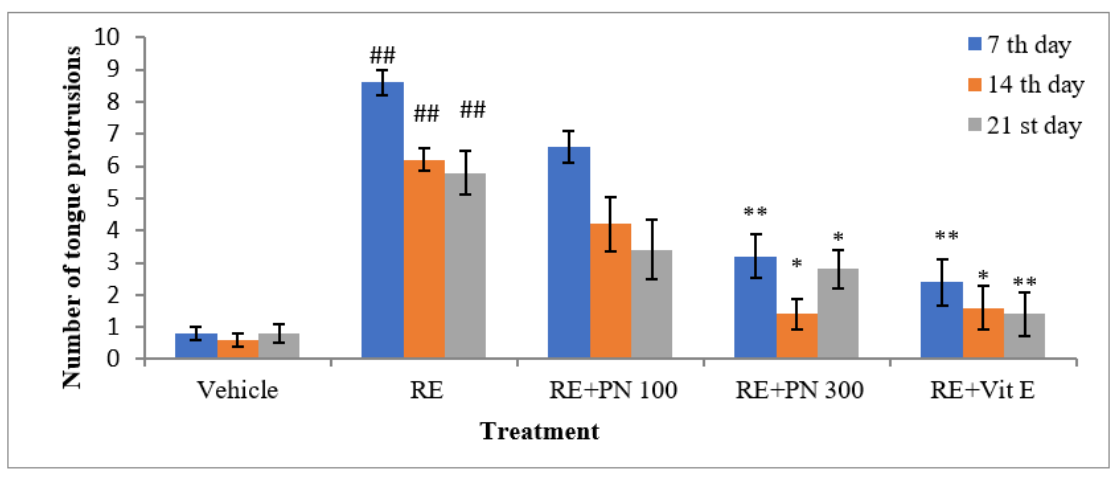

Figure 4: *Effect of P. nigrum on reserpine-induced TPs in rats.*

Grooming

Reserpine treated group exhibited increase grooming in rats. *P. nigrum* significantly reduced number of grooming (Figure 5).

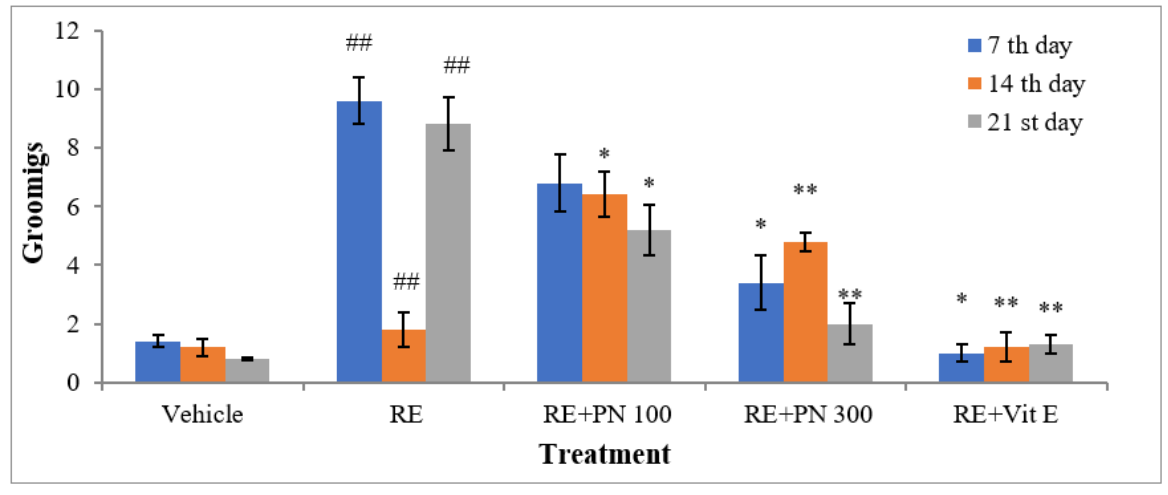

Figure 5: *Effect of P. nigrumon grooming in rats.*

Locomotion

Self-rearing

Reserpine treated group decreases numberof self-rearing in rats. Pretreatment with *P. nigrum* significantly increased number of self-rearing (Figure 6).

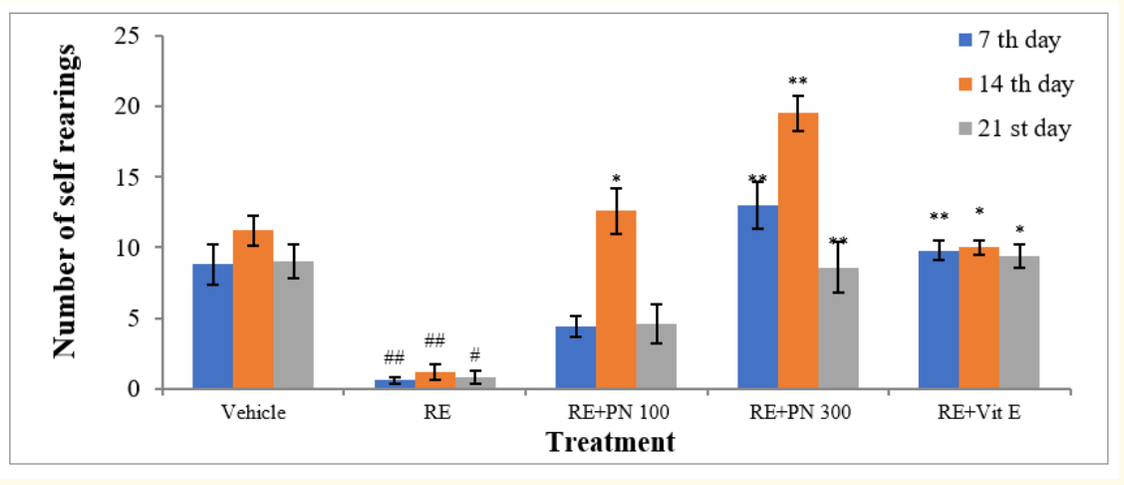

Figure 6: *Effect of P. nigrumon self-rearing in rats.*

Assisted rearing

Animals with Reserpine treatmentshowed decrease innumberof self-rearing. Pretreatment with *P. nigrum* significantly increased number of supported rearing (Figure 7).

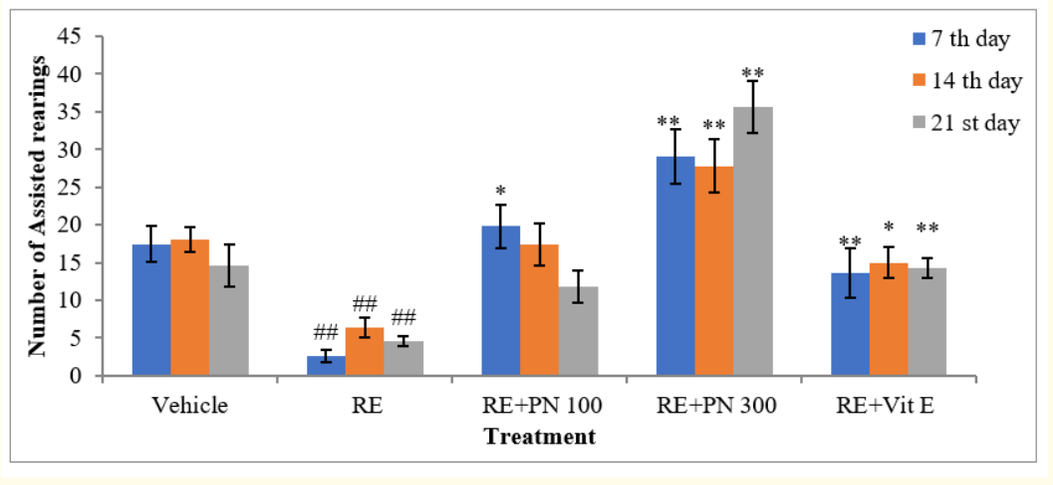

Figure 7: *Effect of P. nigrum onassisted rearing in rats.*

Squares traversed

Animals with Reserpine treatmentdecreases number of squares traversed in rats. Significant rise in number of squares traversed were observed after *P. nigrum*pretreatment compared to reserpine treated rats (Figure 8).

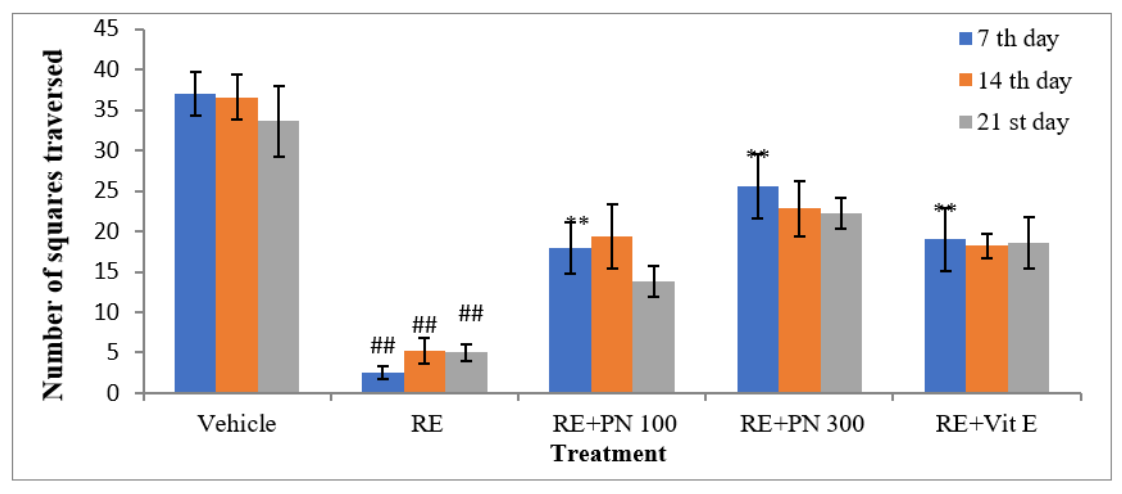

Figure 8: *Effect of P. nigrumon number of squares traversed in rats.*

Cognition

Transfer latency reduces after reserpine treatment. Pretreatment with*P. nigrum* significantly increased transfer latency (Figure 9).

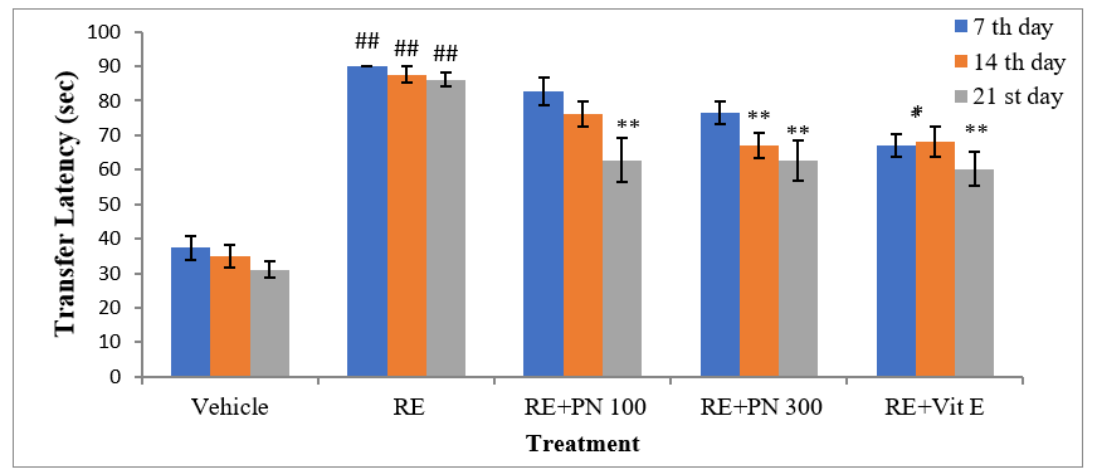

Figure 9: *Effect of P. nigrumon cognition in reserpine treated rats.*

Invivo antioxidant enzymes

Superoxide dismutase (SOD)

Reserpine treatment decreases SOD contentin homogenates of forebrain. Significant reversal in SOD levels were observed after *P. nigrum* and vitamin E compared to reserpine treatment (Figure 10).

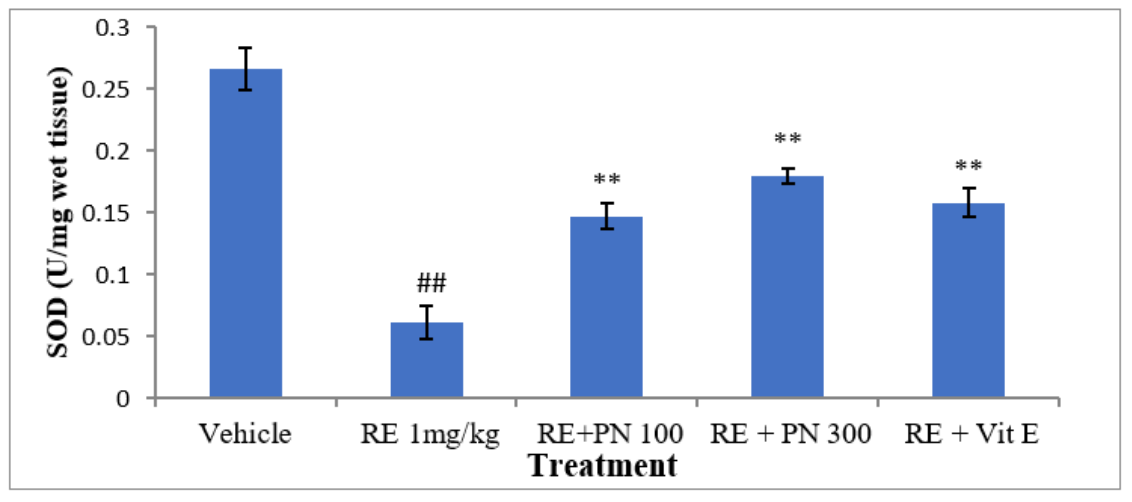

Figure 10: *Effect of P. nigrumon forebrain SOD content in reserpine treated rats.*

Catalase (CAT)

Decrease levels of CAT were observed in reserpine treated animals. Significant reversal in CAT levels were observed after *P. nigrum* and vitamin E compared to reserpine treatment (Figure 11).

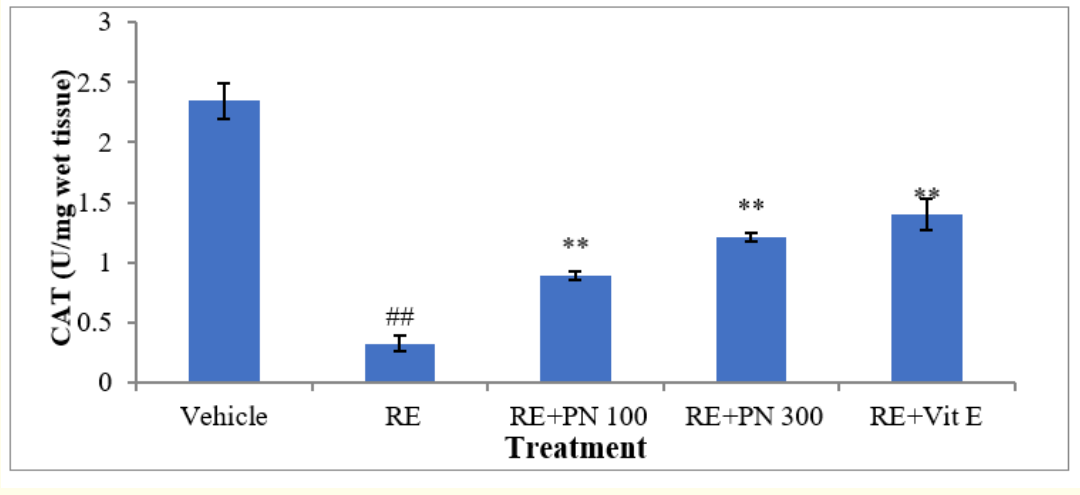

Figure 11: *Effect of P. nigrumon the forebrain CAT content inreserpine treated rats.*

Reduced glutathione (GSH)

Decrease levels of GSH were observed in reserpine treated animals.Significant reversal in GSH levels were observed after *P. nigrum* and vitamin E compared to reserpine treatment (Figure 12).

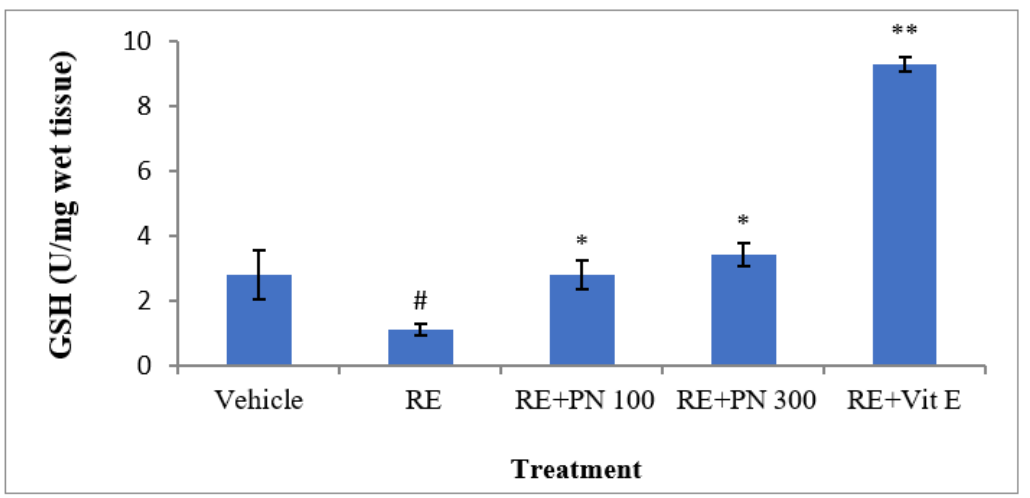

Figure 12: *Effect of P. nigrumon forebrain GSH content in the reserpine treated rats.*

Lipid peroxidative indices (LPO)

Increased levels of MDA are indicative of increased LPO in reserpine treated animals.Significant reversal in extent of lipid peroxidation was observed after*P. nigrum* treatmentalong with reserpinewhen compared to reserpine treated rats (Figure 13).

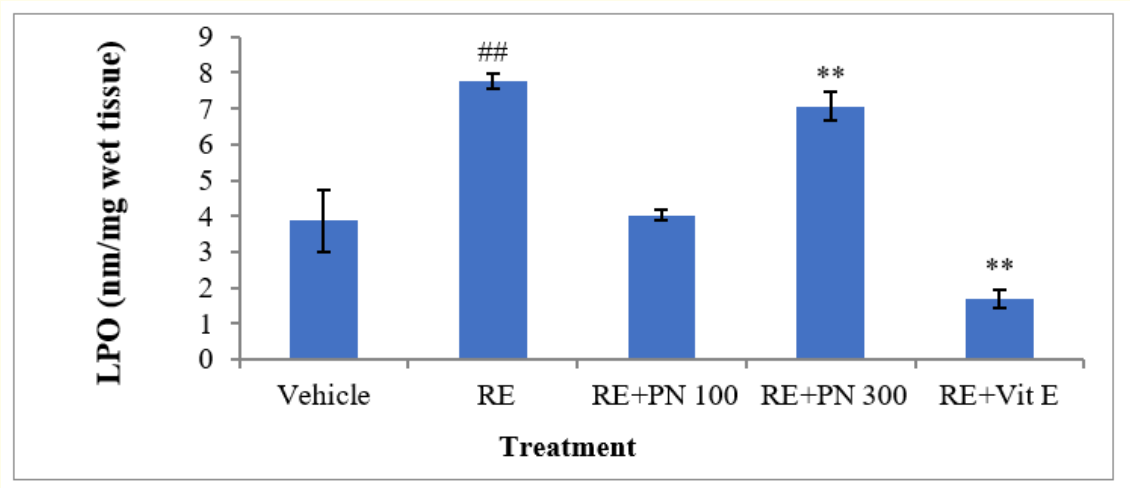

Figure 13: *Effect of P. nigrumon forebrain LPO in reserpine treated rats.*

Discussion

Complex antioxidant system present in human body includes SOD, CAT and GSH act by blocking the initiation of free radical chain reactions [19]. Nonenzymatic antioxidant components include molecules like glutathione, -tocopherol, ascorbic acid, and -carotene, which react with activated oxygen species to inhibit free radical chain reactions from propagating. When free radicals are overproduced or the cellular antioxidant defence mechanism is compromised, they can trigger chain reactions by interaction with proteins, lipids, and nucleic acids, resulting in cellular malfunction or even death.

The use of neuroleptics for a long time increases formation of free radicals and oxidative stress [20]. Increased ROS and oxidative stress have been implicated in the pathogenesis of neuroleptic-induced TD [21,22]. Rats with VCM showed considerably greater TBARS in the striatum, according to Patil and Kasture, implying enhanced lipid peroxidation and free radical generation in these animals [23]. Chronic usage of neuroleptics has also been linked to reduced activity of antioxidant defence enzymes such as SOD and catalase [24].

The brain and nervous system are particularly prone to free radical damage because membrane lipids are quite rich in polyunsaturated fatty acids and certain areas of the brain are quite rich in iron [25-27]. Because they utilise a lot of energy and contain a lot of polyunsaturated fatty acids, the basal ganglia areas of the brain are particularly sensitive to free radical overproduction produced by accelerated dopamine turnover [28].

Reserpine is an antipsychotic medication that has been linked to the development of TD [29,30]. Reserpine-induced OD provides an animal model of TD that has been related to free radical production and oxidative stress based on these analogies.

Antagonism of D_2, M_1, H_1[31] and 5-HT_2c [32,33] receptors located in the hypothalamusmay stimulate appetite. In the present study, sub-acute treatment with *P. nigrum* in reserpine treated animals significantly inhibited weight gain.

Reserpine inhibits monoamine storage, resulting in dopamine, norepinephrine, and serotonin depletion. Low dosages of apomorphine have been shown to reduce dopaminergic transmission by acting on dopamine auto receptors in a reasonably selective manner [34]. Probably dopamine depletion caused by reserpine therapy reduced dopamine release caused by 0.1 mg/kg apomorphine injection, resulting in significant reductions in dopamine release and dramatic increases in VCMs.

Reserpine administration for induction of OD is characterised by increased VCM and TP [10]. Reserpine exhibited development of TD along with increased grooming, which may be suppressed by all anti-PD agents [35]. Sub-acute treatment with *P. nigrum* from day 1 to day 21 along with reserpine significantly and dose-dependently reversed reserpine-induced VCM, TP, OB and decreased the number of grooming.

Reserpine affects the storage of freshly produced dopamine directly (which therefore remains available for metabolism). Treatment with reserpine may result in an increase in dopamine (DA) metabolites and a decrease in dopamine receptor activity. Auto-oxidation or oxidative deamination mediated by monoamine oxidase degrades the DA that is not stored in vesicles or released from nerve terminals (MAO) [36].

To reduce the degree of oxidative stress linked to sickness, several experimental procedures have been employed. Antioxidant supplementation is one method for improving antioxidative defences. By scavenging lipid peroxyl radicals, these compounds inhibit lipid peroxidation. Vitamin E, an endogenous antioxidant and free radical scavenger, is one of the treatments for TD [10].

A noteworthy reversal of decreased levels of SOD and CAT in reserpine treated animals was observed after *P. nigrum* treatment. Flavonoids act as scavengers of ROS [37].

As per phytochemical analysis, crude ethanolic extract included alkaloids, glycosides, tannins, and flavonoids. Several of these chemicals have been shown to have strong antioxidant properties. Some of the components in this plant have already been separated. Because of the presence of phytochemical ingredients, particularly polyphenols, antioxidant activity in ethanolic extracts of *P. nigrum* indicates increased free radical scavenging activity.

Results of this study are compatible with the hypothesis of Patil., *et al.* [10] and Patil and Kasture [23], who postulated that oxidative stress act as major contributor in the development of OD.

Conclusion

The findings of the present study strongly suggest the role of oxidative stress in the pathophysiology of neuroleptic-induced orofacial dyskinesia, behavioural and biochemical changes. We have demonstrated that *Piper nigrum*is good scavenger of oxygen species and attenuates the behavioural and biochemical changes induced by administration of reserpine and are useful for prevention or treatment of orofacial dyskinesia**.** These fruits can be used in pharmaceutical industries as natural antioxidants.

Conflict of Interest

None.

Financial Support

None.

Ethics Statement

None.

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