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Ethanol extract of *Epipremnum aureum* leaves attenuate intranigral-rotenone induced Parkinson's disease in rats

[El extracto etanólico de hojas de *Epipremnum aureum* atenúa la enfermedad de Parkinson inducida por rotenona intranigral en ratas]

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Abstract

Context: Parkinson's disease (PD) is characterized by motor-symptoms attributed to profound (60 - 70%) neurodegeneration in the striatum and loss of dopamine thereof. Oxidative stress, mitochondrial dysfunction and neuroinflammation are major contributors to neurodegenerative pathogenesis of PD. *Epipremnum aureum* is an ornamental plant found to possess significant antioxidant and anti-inflammatory properties.

Aims: To evaluate the neuroprotective potential of ethanol extract of *E. aureum* (EEA) leaves against rotenone-induced neurotoxicity in rats.

Methods: Stereotaxic brain surgery was performed on day 1 and rotenone (ROT) was injected (300 µg/µL) in the substantia nigra pars compacta (SNpc) to induce PD in rats. EEA (125, 250 and 500 mg/kg, p.o.) was administered to ROT-treated rats for 28 consecutive days. Rearing behavior, ambulation, catalepsy and paw retraction test were performed on day 28. After behavioral tests, rats were sacrificed, whole-brain removed and TBARS, GSH and catalase activity was determined in brain homogenate.

Results: ROT-treated rats showed a conspicuous increase in the catatonic score, paw retraction time and decline in the ambulatory score and rearing score that exhibited induction of PD in rats. The motor-symptoms triggered by rotenone were significantly reversed in EEA (250 and 500 mg/kg) treated animals. EEA treatment significantly attenuated the intranigral ROT induced rise in brain TBARS and a decline in GSH content and catalase activity.

Conclusions: The study demonstrated that in the present model of PD, treatment with EEA mitigates the key symptoms (e.g. hypokinesia, rigidity and postural instability) through potent antioxidant activity.

Keywords: catalepsy; *Epipremnum aureum*; oxidative stress; Parkinson's disease; rotenone; substantia nigra pars compacta.

Resumen

Contexto: La enfermedad de Parkinson (EP) se caracteriza por síntomas motores atribuidos a una neurodegeneración profunda (60-70%) en el cuerpo estriado y la pérdida de dopamina del mismo. El estrés oxidativo, la disfunción mitocondrial y la neuroinflamación son los principales contribuyentes a la patogénesis neurodegenerativa de la EP. *Epipremnum aureum* es una planta ornamental que posee propiedades antioxidantes y anti-inflamatorias significativas.

Objetivos: Evaluar el potencial neuroprotector del extracto etanólico de *E. aureum* (EEA) contra la neurotoxicidad inducida por rotenona en ratas.

Métodos: La cirugía cerebral estereotáxica se realizó el día 1 y se inyectó rotenona (ROT) (300 µg/µL) en sustancia negra compacta (SNpc) para inducir la EP en ratas. Se administró EEA (125, 250 y 500 mg/kg, p.o.) a ratas tratadas con ROT durante 28 días consecutivos. La prueba de comportamiento de crianza, la deambulación, la catalepsia y la retracción de la pata se realizaron el día 28. Después de las pruebas de comportamiento, se sacrificaron las ratas, se extrajo todo el cerebro y se determinó la actividad de TBARS, GSH y catalasa en homogeneizado de cerebro.

Resultados: Las ratas tratadas con ROT mostraron un notable aumento en la puntuación catatónica, el tiempo de retracción de la pata y la disminución en la puntuación ambulatoria y la puntuación de crianza que exhibieron inducción de EP en ratas. Los síntomas motores desencadenados por la rotenona se revirtieron significativamente en los animales tratados con EEA (250 y 500 mg/kg). El tratamiento con EEE atenuó significativamente el aumento inducido por ROT intranigral en los TBARS cerebrales y la disminución en el contenido de GSH y la actividad de catalasa.

Conclusiones: El estudio demostró que, en el presente modelo de EP, el tratamiento con EEA mitiga los síntomas clave (por ejemplo, hipocinesia, rigidez e inestabilidad postural) a través de una potente actividad antioxidante.

Palabras Clave: catalepsia; *Epipremnum aureum*; estrés oxidativo; enfermedad de Parkinson; rotenona; sustancia nigra pars compacta.

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INTRODUCTION

Parkinson's disease (PD) is second most prevalent progressive neurodegenerative disorder that primarily involves loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) and dopamine deficiency in the striatum (Sveinbjornsdottir, 2016). Intraneuronal accumulation of *a*-synuclein and ubiquitin in intracytoplasmic inclusions known as Lewy bodies (LB) at brainstem, olfactory area, limbic and neocortical regions of the brain are the major neuropathological features of PD (Meredith et al., 2008). PD affects 1-3% of people with age more than 65 years depicting a high prevalence in males with respect to female counterparts. Degeneration of 70% neurons in SNpc emanates motor-symptoms such as bradykinesia, rigidity, tremor, and deformity of balance, posture and gait in PD. The non-motor symptoms preceding long before motor-symptoms are constipation, sleep disturbances (e.g. hyposmia, rapid eye movement), urinary incontinence, hallucinations, dementia and hypotension (Radhakrishnan and Goyal, 2018). The sporadic form of PD is more common in comparison to familial (10%) PD (Moore et al., 2005). The genetic basis of PD includes several genes such as *Parkin*-2, *Leucine rich repeat kinase-2*, *a-synuclein*, *PTEN induced putative kinase-1*, *DJ1*, *ubiquitin C-terminal hydrolase like-1*, and *ATPase type 13A2*. Modern imaging techniques such as SPECT, PET and FP-CIT are currently used to assess the density of dopaminergic neurons, monoaminergic disturbances and dopamine transporter (DAT) functions in the brain regions (Radhakrishnan and Goyal, 2018).

Several evidences substantiate the role of oxidative stress and neuroinflammation in appearance of behavioral and biochemical alterations in PD (Dauer and Przedborski, 2003). Oxidative stress induced mitochondrial abnormalities are specifically related with PD. It is observed that chronic exposure to chemicals such as paraquat (herbicide) and maneb (fungicide) instigate the pathology of PD through reactive oxygen species (ROS). Mitochondrial dysfunction further potentiates the free radical generation that forms a vicious cycle of self-replenishing neurotoxic agents. Dysfunction of complex I of the mitochondrial electron transfer chain (ETC) by rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-Methyl-4-phenylpyridinium (MPP+), paraquat and maneb (manganese ethylenebisadithiocarbamate) induce PD symptoms in rodents (Sherer et al., 2003). The dopaminergic neurons of SNpc are vulnerable to these selective inhibitors of the electron transport chain (e.g. MPTP and rotenone) that enable the use these agents for *in vitro* and *in vivo* experimental models of PD (Meredith et al., 2008). The ROS mediated lipid peroxidation generates secondary neurotoxic messengers (e.g. malondialdehyde, 5-hydroxynonenal, isoprostanes) that are highly immunogenic. Activation of microglia and astrocytes give rise to pro-inflammatory cytokines such as TNF-*a*, IL-1, IL-6 and nitric oxide. Many *in vivo* animal studies have validated chronic elevation of the brain pro-inflammatory cytokines, activation of iNOS, COX-2 and microgliosis in PD (Zaitone et al., 2012). The emergence of inflammation-based animal models (e.g. intracerebral and systemic lipopolysaccharide; 5 ng-5 mg/kg) displays the devastating effect of neuroinflammatory cascade in PD (Meredith et al., 2008).

At present the pharmacotherapy of the motor-symptoms include levodopa/carbidopa, monoamine oxidase-B inhibitors (e.g. selegiline andrasagiline), ergotamine derived dopamine agonists (e.g. bromocriptine, lisuride and cabergoline), non-ergotamine derived dopamine agonists (e.g. pramipexole, ropinirole, apomorphine), catechol-*O*-methyltransferase (COMT) inhibitors (e.g. entacapone and tolcapone), *N*-methyl-*D*-aspartate (NMDA) receptor inhibitors, and anti-cholinergics (e.g. trihexyphenidyl and benztropine) (Abeliovich and Gitler, 2016). These aforementioned drugs provide symptomatic relief to patients of PD at early stages only with limited long-term benefits and are unable to reverse the pathogenic neurodegenerative progression of disease. There are several potential anti-Parkinson drugs currently under clinical trials (e.g. Safinamide in phase III, caffeine, coenzyme-Q, 17β-estradiol, GM-I ganglioside, nicotine) that can slow-down the spread of

PD (Ravina et al., 2003). However, there is dearth of an effective pharmaceutical agent that can attenuate the progression of pathology of PD.

Epipremnum aureum (Linden & André) G.S. Bunting (*Araceae*) is an evergreen herbaceous shrub commonly used as popular ornamental foliage (golden pothos; money plant) that originated from Southeast Asia and the Solomon Islands in Indonesia (Meshram and Srivastava, 2014). *E. aureum* is traditionally used in cancer and diverse skin diseases in may countries such as Malaysia and Singapore (Das et al., 2017). *E. aureum* is explored to possess several pharmacological activities such as analgesic, antibacterial, anti-inflammatory, antifungal, antioxidant, detoxifying and hypoglycemic (Meshram and Srivastava, 2015; Abhinayani et al., 2016). In previous studies, gas chromatography-mass spectrometry analysis (GC-MS) showed the presence of thirty compounds out of which ten are known to have biological activity. The leaves are reported to have hexadecanoic acid methyl ester, squalene, flavonoids and phenols that exhibit many of the beneficial bioactivities (Linnet et al., 2010; Arulpriya and Lalitha, 2011; Sonawane et al., 2011). Neuroprotective effect of the phenolic content (e.g. caffeic acid, ferulic acid and rosmarinic acid) of *E. aureum* against 6-OHDA lesioned nigral dopamine neurons has been noted (Kurauchi et al., 2012; Ojha et al., 2015). This research aimed to explore the effect of ethanol extract of *E. aureum* leaves in experimental model of rotenone-induced PD in rats.

MATERIAL AND METHODS

Experimental animals

The research protocol was approved by the Institutional Animal Ethics Committee of the institute (Protocol approval no. ASCB/IAEC/08/16/110, date: 24-09-2016). Wistar rats (220-250 g) were procured from the Central Animal Facility, All India Institute of Medical Sciences (New Delhi). The care of laboratory animals was done following the guidelines of CPCSEA, Ministry of Forests & Environment, Government of India. Animals were housed in polypropylene cages with dust free rice husk as a bedding material and maintained under standard laboratory conditions with controlled temperature (23 ± 2°C), humidity (40 ± 10%) and light-dark cycle (12 h each). The animals were fed with a standard rodent pellet diet (Ashirwad Industries, Mohali) and water *ad libitum*. The animals were acclimatized for seven days to the housing conditions of the Central Animal House Facility of the institute prior to experiments. The experiments were carried out between 09:00 and 18:00 h.

Drugs and chemicals

Rotenone (Sigma Aldrich); pramipexole (Pramipex®, SunPharma Labs); sodium dodecyl sulfate, acetic acid, pyridine, hydrogen peroxide and Folin's Ciocalteau reagent (LobaChemie, Mumbai); thiobarbituric acid, dimethylsulfoxide (DMSO), reduced glutathione (Himedia, Mumbai); *n*-butanol (Merck, Mumbai); chlorhexidine gluconate (Hexiprep®, IPCA, Mumbai); meloxicam (Melonex®, Intas Pharma, India) and phenobarbital sodium (Nicholas Piramal, India) were used. All the other chemicals were of analytical grade.

Plant material

The leaves of *Epipremnum aureum* were collected from a garden in Ropar, Punjab (GPS 30.967561, 76.519004) in mid of rainy-autumn season (September 2016). A voucher specimen of the plant leaves was verified by Dr. A.S. Sandhu, National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab (India).

Preparation of *Epipremnum aureum* leaves extract

The leaves of *Epipremnum aureum* were washed with fresh water, dried in shade for five days and then coarsely powdered with the help of mechanical grinder (SONAR, Delhi, India). The powder (particle size <1.8 mm; sieve no. 10/44) was stored in airtight glass container for further use at temperature 20 - 23°C. The powdered leaves (100 g) were packed in Soxhlet apparatus and de-fatted with petroleum ether (1000 mL) (40 - 60°C) for 72 h, allowed to dry and then extracted (94 g) using ethanol (1000 mL) as a solvent in Soxhlet apparatus. The temperature (55°C) was maintained on an electric heating mantle with thermostat control

(INCO, Ambala, India). The appearance of colorless solvent in the siphon tube depicted completion of extraction. The extract was concentrated to syrupy consistency on water bath. The concentrated extract was then air dried at room temperature and stored in airtight container at 2 - 8°C until used (Das et al., 2015). The yield obtained using ethanol Soxhlet extraction was found to be 9.4%.

Administration of rotenone in substantia nigra pars compacta of rat brain

Rats were anesthetized using phenobarbital (60 mg/kg; i.p.) and toe-pinched to check the degree of anesthetization. Afterwards, the head area from the ears to just in between the eyes was shaved using an electric razor (BT3221/15® Philips, India). The head was fixed in the stereotaxic frame (INCO, Ambala, India) using ear bars while the nose bar was used to fix the mouth of the animal. Chlorhexidine (0.4% w/v) was applied over shaved area and then an anterior-posterior incision of about 2.5 cm was made with the help of the sterile surgical blade midline of the scalp from center of eyes up to the back of the ears. Bulldog clamps were used to expose the skull and bregma was located. A burr hole was made in the skull using coordinates (antero-posterior from bregma 5.0 mm; mediolateral from midsagittal suture 2.0 mm; dorso-ventral from skull 8mm) for the left substantia nigra pars compacta. Rotenone (ROT; 3 µg/µL dimethylsulfoxide) was slowly injected using Hamilton® microsyringe. Post-rotenone injection the hole was closed with dental cement, wound was cleaned with sterile saline and skin sutured. After the surgery the animal was transferred into a recovery cage and monitored up to 4 days. Surgery was performed using a heating pad (Electric Heating Pad, Bhagwati Pharma Surgicals, Vadodra, India) and post-surgical hypothermia was avoided by maintaining the temperature 37 ± 0.5°C. The animals were given meloxicam (2 mg/kg, p.o.) to minimize the post-operative pain (Erbas et al., 2012).

Experimental protocol

Thirty-six rats were divided into six groups (n=6) and subjected to brain surgery on day 1. Parkinson's disease was induced in rats by slow intranigral injection of rotenone (3 µg/µL). The rotenone administered groups were further divided into ROT, EEA-125, EEA-250, EEA-500 and Pramipexole (PPX) groups. Ethanol extract of *Epipremnum aureum* (EEA) was administered (doses 125, 250 and 500 mg/kg body weight; p.o.) to separate groups of rotenone-treated rats from day 1 to day 28 (Das et al., 2015; Abhinayani et al., 2016). PPX, dopamine agonist, served as standard drug and administered at dose 2 mg/kg b.w. (p.o.) for 28 consecutive days from day 1. ROT group received intranigral rotenone on day 1 and normal saline for 28 days. DMSO group served as control and was given intranigral injection of DMSO vehicle (1 µL) only on the first day. Behavior parameters (rearing, catalepsy, open field test and the paw test) were tested on day 27 and 28. After behavioral studies, the animals were sacrificed by decapitation for estimating thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) levels and catalase activity in the whole brain (Fig. 1).

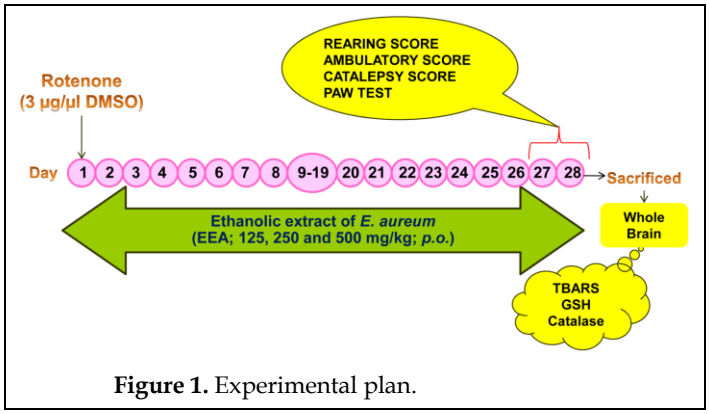

Figure 1. Experimental plan.

Rearing behavior

The rearing behavior was assessed using the open field apparatus (INCO, Ambala, India) that comprised of a circular enclosure 30 cm high secured to a plywood base 92 cm in diameter. The number of rears were quantified, and each rear was counted as a rearing score. The rise of forelimbs by animal above the shoulder level to make contact with the cylinder wall with either one or both forelimbs was counted as rearing. Removal of both forelimbs from the cylinder wall and contact with the table surface was required before another rear to be scored. This test has been successfully used previously to assess behavioral deficits in rats receiving rotenone (Fleming et al., 2004).

Ambulation behavior

The open-field consisted of a circular enclosure (height 30 cm) firmly attached to a plywood base (diameter 92 cm) (INCO, Ambala, India). The enclosure and the base painted white with the base having three concentric circles marked on it in black. These circles are subdivided by radiating black lines into 25 segments of equal area. Each animal was placed in the innermost circle of the open field and observed for the locomotor activity by assessing ambulation. Ambulation is defined as the number of lines crossed by animal with all of its paws (Bond and Giusto,1976).

Catalepsy test

The scoring method adopted in this test consists of three steps. In **s**tep I, the rat was taken out of the home cage and placed on a table. If the rat failed to move when touched or pushed gently on the back a score of 0.5 was assigned. In **s**tep II the front paws of the rats were placed alternately on a 3-cm high block. If the rat failed to correct the posture within 15 seconds, a score of 0.5 for each paw was added to the score of step I. In **s**tep III the front paws of the rat were placed alternately on a 9-cm high block, if the rat failed to correct the posture within 15 seconds a score of 1 for each paw was added to the cumulative scores of steps I and II. Thus, the highest score for any animal was 3.5 (cut off score) that reflected the catalepsy (Rasheed et al., 2010).

The paw test

The test apparatus consisted of a wooden frame with four holes, two holes of 4 cm diameter for the forelimbs and two holes of 5 cm for the hindlimbs. The rat was released free as soon as the rat is placed with all four limbs in the holes. The latency to retract the first fore and hind-limbs i.e. fore-limb retraction time (FRT) and hind-limb retraction time (HRT) from the holes was noted with 30 s cut-off time. Because of the difficulty in establishing the exact starting time of the retraction effects, the minimum time was set to 1 s. Thus, when rats immediately retracted one forelimb from a hole in the paw test, the forelimb retraction time was counted as 1 s. The reduction in the FRT and HRT exhibited anti-Parkinsonian effect (Ellenbroek et al., 1987; Vrijmoed-de Vries et al., 1987).

Brain tissue homogenate preparation

After behavioral studies, the animals were sacrificed (n=6) by cervical dislocation, whole brains were removed and instantly placed on ice followed by rinsing with ice-cold isotonic saline (0.9% NaCl) to remove the residues and blood, and whole brain wet weight was noted down. Brain tissue homogenate (10% w/v) was prepared in 50 mM phosphate buffer (pH 7.4; temperature 4°C) using tissue homogenizer (Remi Motors, Remi Electrotechnik, Vasai, India). The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C in high speed refrigerated centrifuge (CPR-30 Remi Compufuge, India) and supernatant was separated from sediment for estimation of biochemical parameters.

Estimation of thiobarbituric acid reactive substances

Measurement of thiobarbituric acid reactive substances (TBARS) is a reliable index of lipid peroxidation product malondialdehyde (pink color MDA-TBA_2 adducts). The 4 mL assay mixture consisted of 0.1 mL homogenate, 0.2 mL sodium dodecyl sulphate (8.1%), 1.5 mL glacial acetic acid (20%, pH 3.5), 1.5 mL TBA (0.8%) and 0.7 mL dis-

tilled water. Tubes were thoroughly mixed, heated at 95°C for 1 h on a water bath, cooled under tap water, vigorously mixed with 5 mL mixture of n-butanol and pyridine (15:1) and centrifuged at 4000 rpm for 10 min. The absorbance of upper 2 mL organic layer (*n*-butanol phase) was measured at a wavelength of 532 nm using spectrophotometer (Shimadzu UV-1700, Pharmaspec). The amount of MDA formed or TBARS was calculated by using molar extinction coefficient of chromophore 1.56×10^5 M^{-1} cm^{-1} and expressed as micromole TBARS/mL (Ohkawa et al., 1979).

Estimation of brain GSH level

The 1 mL supernatant was precipitated with 1 mL sulphosalicylic acid (4%), cold digested for 1 h at 4°C and after 5 min centrifuged at 2000 rpm for 10 min at 4°C. To 0.1 mL of the supernatant obtained, 2.7 mL phosphate buffer (0.3 M, pH 8) and 0.2 mL DTNB (0.1 mM, pH 8) was added. The absorbance was measured at 412 nm using spectrophotometer (Shimadzu UV-1700, Pharmaspec) and amount of GSH (micromole GSH/mL) calculated using molar extinction coefficient 1.36×10^4 M^{-1} cm^{-1} of the chromophore (Ellman, 1959).

Assay for catalase activity

The assay mixture consisted of 1.95 mL phosphate buffer (0.05 M, pH 7), 1 mL hydrogen peroxide (0.02 M, prepared in 0.05 M phosphate buffer) and 0.05 mL supernatant (10%) in a final volume of 3 mL. Change in absorbance for 3 min at 30 seconds interval was recorded at 240 nm using spectrophotometer. Enzymatic activity was calculated using molar extinction coefficient of 43.6 M^{-1} cm^{-1} at 240 nm and reported as micromole H_2O_2 decomposed/min/mL (Claiborne, 1985).

Statistical analysis

All the results are expressed as mean ± S.E.M. The data of all the groups were analyzed by one-way ANOVA followed by Tukey's *post-hoc* test using software Graph Pad Prism 5 (Graph Pad Software Inc., USA). A value of $p<0.05$ was considered significant.

RESULTS

Ethanol extract of *E. aureum* leaves increased rearing behavior in intranigral-rotenone treated rats

The ROT group showed a significant reduction ($p<0.001$) in rearing score as compared to the DMSO group. Administration of EEA (250 and 500 mg/kg; p.o.) to rats previously treated with ROT significantly increased ($p<0.05$ and $p<0.01$, respectively) the number of rearings as compared to the rearing score of ROT alone treated rats. The present findings corroborated reversal of ROT induced rearing behavior by EEA in rats. Administration of pramipexole (PPX), dopamine agonist, to ROT treated rats also improved ($p<0.001$) the rearing score as compared to the animals that received ROT alone (Fig. 2A).

Ethanol extract of *E. aureum* leaves attenuated the decline of ambulation scores in intranigral-rotenone treated rats

The ambulation score was conspicuously reduced ($p<0.001$) in the ROT group as compared to the DMSO group. EEA-250 and EEA-500 groups exhibited increase ($p<0.05$ and $p<0.01$, respectively) in ambulation score as compared to ROT group. Pramipexole (PPX) has proven efficacy in patients of PD and was used as standard drug in current protocol. Treatment with pramipexole increased ($p<0.001$) the ambulation scores of rotenone treated rats in comparison to rats that received ROT alone (Fig. 2B).

Ethanol extract of *E. aureum* leaves abolished intranigral-rotenone induced rise in catalepsy scores of rats

The catalepsy score was significantly increased ($p<0.001$) in the animals of the ROT group as compared to the DMSO group. EEA-500 group showed significant ($p<0.05$) decrease in catalepsy in comparison to ROT group. The treatment of rotenone injected rats with pramipexole (PPX) also displayed reduction ($p<0.01$) in catalepsy score with respect to rats that received ROT alone (Fig. 2C).

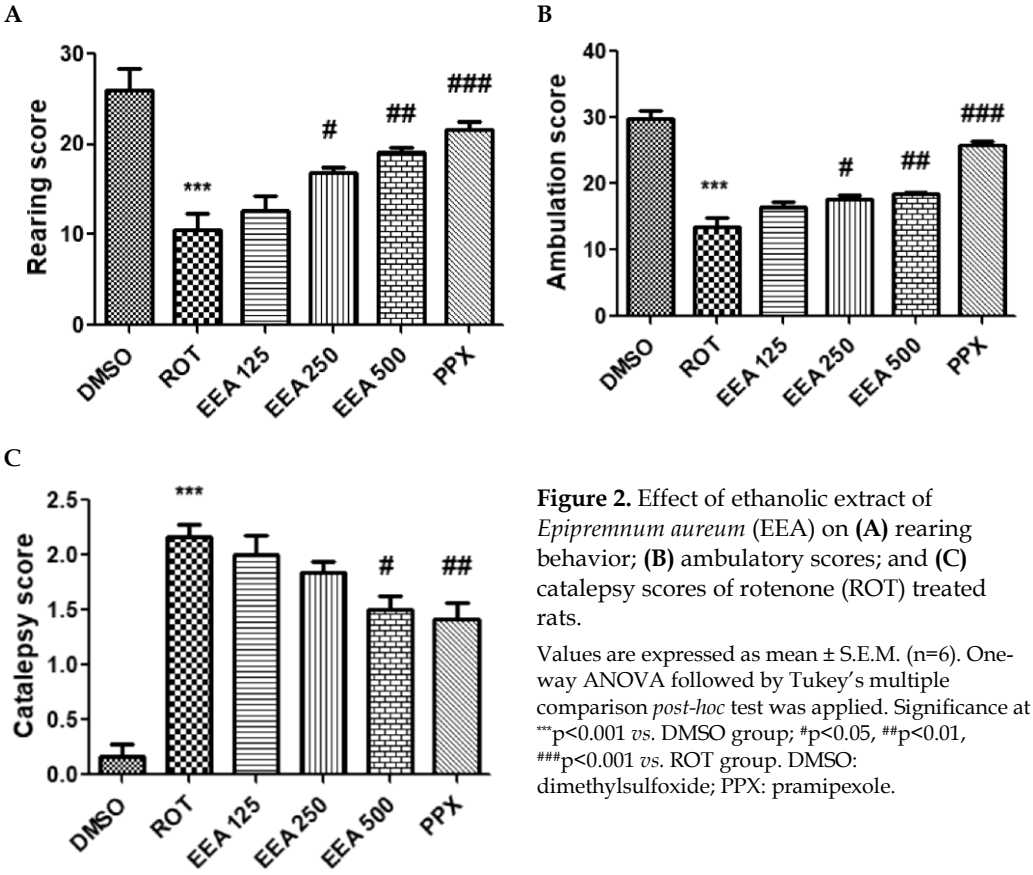

Figure 2. Effect of ethanolic extract of *Epipremnum aureum* (EEA) on **(A)** rearing behavior; **(B)** ambulatory scores; and **(C)** catalepsy scores of rotenone (ROT) treated rats.

Values are expressed as mean ± S.E.M. (n=6). One-way ANOVA followed by Tukey's multiple comparison *post-hoc* test was applied. Significance at ***p<0.001 *vs.* DMSO group; #p<0.05, ##p<0.01, ###p<0.001 *vs.* ROT group. DMSO: dimethylsulfoxide; PPX: pramipexole.

These results depicted amelioration of ROT triggered symptom of PD (catalepsy) by EEA in rats.

Ethanol extract of *E. aureum* leaves reduced paw retraction time in intranigral-rotenone treated rats

Intranigral administration of ROT in rats significantly (p<0.001) increased the forelimb retraction time (FRT) and hind-limb retraction time (HRT) as compared to the rats that received DMSO vehicle only. The treatment of rotenone injected rats with EEA (125 and 250 mg/kg) exhibited modest decline (p>0.05) in paw retraction time as compared to rats that received rotenone alone. However, oral administration of EEA (500 mg/kg) to rotenone treated rats profoundly reduced (p<0.05) both FRT and HRT with respect to rats that received rotenone alone. PPX group showed reduction (p<0.01) in the paw retraction time as compared to the ROT group (Fig. 3).

Ethanol extract of *E. aureum* leaves attenuated intranigral-rotenone induced rise in the brain TBARS level

Intranigral administration of rotenone (3 µg/µL) in rats increased (p<0.001) the brain TBARS levels as compared to administration of DMSO vehicle alone. EEA-250 and EEA-500 groups exhibited significant reduction (p<0.05 and p<0.01, respectively) in TBARS levels as compared to ROT group. The PPX group displayed significant reduction (p<0.001) in brain TBARS level as compared to ROT group (Fig. 4A). These findings showed attenuation of lipid peroxidative changes by EEA in ROT treated rats.

Ethanol extract of *E. aureum* leaves enhanced the brain GSH level in intranigral-rotenone treated rats

The administration of ROT in substantia nigra area of rat brain markedly depleted (p<0.001) the

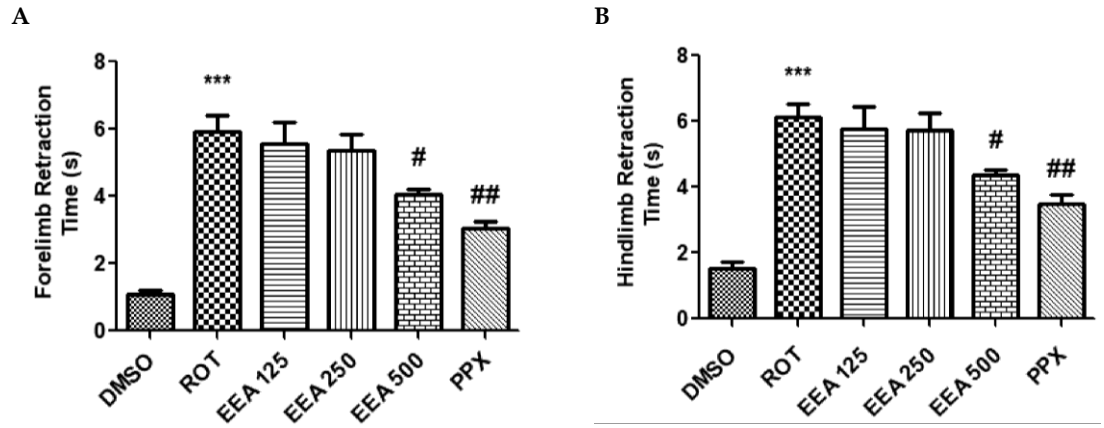

Figure 3. Effect of ethanolic extract of *Epipremnum aureum* (EEA) on paw retraction time of rotenone (ROT) treated rats.

Values are expressed as mean ± S.E.M. (n=6). One-way ANOVA followed by Tukey's multiple comparison *post-hoc* test was applied. Significance at ***p<0.001 *vs.* DMSO group; #p<0.05, ##p<0.01 *vs.* ROT group.

DMSO: dimethylsulfoxide; PPX: pramipexole.

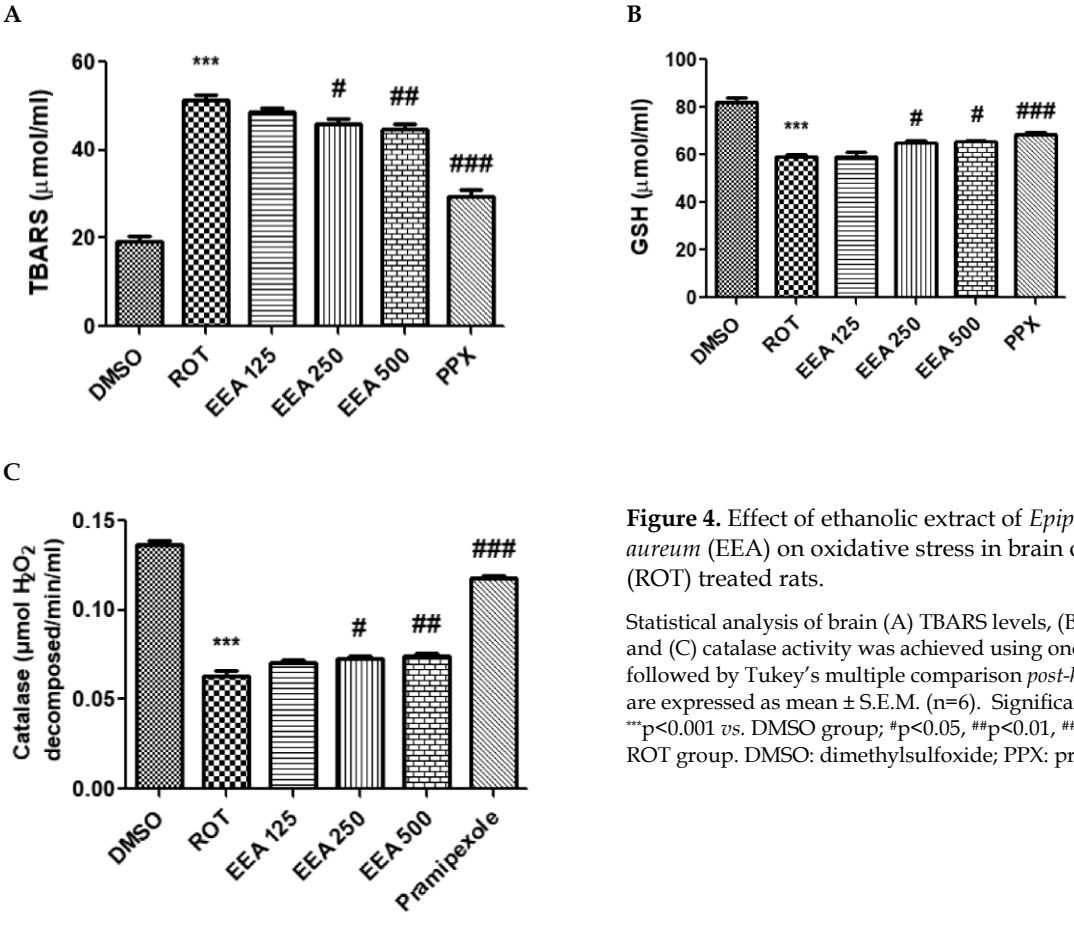

Figure 4. Effect of ethanolic extract of *Epipremnum aureum* (EEA) on oxidative stress in brain of rotenone (ROT) treated rats.

Statistical analysis of brain (A) TBARS levels, (B) GSH content, and (C) catalase activity was achieved using one-way ANOVA followed by Tukey's multiple comparison *post-hoc* test. Values are expressed as mean ± S.E.M. (n=6). Significance at ***p<0.001 *vs.* DMSO group; #p<0.05, ##p<0.01, ###p<0.001 *vs.* ROT group. DMSO: dimethylsulfoxide; PPX: pramipexole.

GSH levels as compared to administration of DMSO vehicle alone. EEA-250 and EEA-500 groups showed significant improvement ($p<0.05$) in GSH levels with respect to ROT group. The PPX group displayed significant enhancement ($p<0.001$) in brain GSH level as compared to ROT group (Fig. 4B). The present findings demonstrated that EEA fortified the endogenous antioxidant defense in the brain of rats against ROT-neurotoxicity.

Ethanol extract of *E. aureum* leaves elevated the brain catalase activity in intranigral-rotenone treated rats

The brain catalase activity was significantly decreased ($p<0.001$) in rotenone alone administered group as compared to DMSO treated group. EEA-250 and EEA-500 groups exhibited significant enhancement ($p<0.05$ and $p<0.01$ respectively) in brain catalase activity as compared to ROT group. The PPX group displayed significant rise ($p<0.001$) in brain catalase activity as compared to ROT group (Fig. 4C). The present findings indicated that EEA effectively abolished the ROT-induced decline in antioxidant activity in the brain of rats.

DISCUSSION

Parkinson's disease is characterized by selective degeneration of dopaminergic neurons in the SNpc. Although the understanding of pathogenesis of PD is limited to accumulation of Lewy bodies in striatal neurons, loss of dopaminergic and increase in cholinergic activity, however, mitochondrial dysfunction, oxidative damage and apoptosis have been the major contributing factors. Exposure to pesticides, metals and/or solvents is one of the major causes of the development of PD in modern world (Meredith et al., 2008). Rotenone (ROT) is a commonly used pesticide that induce key symptoms of PD in experimental rodents, when administered systemically. The growing body of evidence suggests that the ROT model of PD offers more advantages than the other experimental models as it can effectively mimic the behavioral and neuropathological features (e.g. oxidative stress, aggregation of *a*-synuclein and Lewy bodies) of the disease (Erbas et al., 2012). Owing to its high lipophilicity, rotenone can enter cell organelles including mitochondria, and inhibits complex-I (NADH:ubiquinone oxidoreductase) activity in the mitochondrial ETC. The inhibition of complex-I culminates in higher ROS output, ATP reduction and cell death in SNpc leading to manifestation of PD characteristics. Recently, the high morbidity and mortality rate in experimental subjects associated with systemic rotenone treatment ushered to stereotaxic administration of rotenone directly in SNpc of rodents to eliminate high systemic toxicity. Hence, intranigral rotenone (3 µg/µL) lesion rat model was used in this study to examine the neuroprotective and/or neurorepair potential of ethanol extract of *Epipremnum aureum* (EEA) leaves (125, 250 and 500 mg/kg) in PD.

In the present study, rotenone (ROT) precipitated the motor-symptoms evident by increase in the catatonic score, paw retraction time and decline in the ambulatory score and rearing score that exhibited induction of PD in rats. Characteristic features of PD like hypokinesia, postural instability and freezing were also observed in the rats that received ROT alone. The motor-symptoms triggered by rotenone were significantly reversed in EEA (250 and 500 mg/kg) treated animals. Pramipexole is a dopamine agonist and clinically used drug for PD. In the current model of PD, pramipexole significantly abolished the ROT induced motor-symptoms of PD.

ROT is a naturally occurring compound (*Lonchocarpus* and *Derris* species) that inhibits the complex I of mitochondrial respiratory chain. ROT inhibits transfer of electrons from the NADH (Fe-S) to ubiquinone or coenzyme-Q that blocks oxidative phosphorylation and subsequent synthesis of ATPs. Incomplete electron transfer to molecular oxygen (O_2) amplifies the ROS output from mitochondria. ROS are detrimental to mitochondrial membranes and DNA (mtDNA) that corroborates apoptosis in neurons (Heinz et al., 2017). Some studies also depicted inhibition of mitosis and cell proliferation *via* blockade of microtubular assem-

bly formation by rotenone (Cappelletti et al., 2015). These structural abnormalities disturbs the neuronal polarity, axonal transport and synaptic plasticity which amplify progression of PD. Mitochondrial dysfunction, abnormalities in complex I activity and high ROS have been observed in the brain of PD patients and are associated with severe PD motor-symptoms (Schapira, 2008). The whole brain analysis revealed that rotenone administration in rats enhanced the oxidative stress in brain depicted by increased TBARS, and reduced GSH levels and catalase activity. TBARS is a reliable indicator of free radical initiated lipid peroxidation product malondialdehyde (MDA). MDA is the most neurotoxic secondary mediator that forms highly immunogenic adducts with biomolecules and is widely used as biomarker of oxidative stress. In acidic environment the MDA-acetaldehyde adduct, and MDA-advanced glycation end product adducts are overtly mutagenic and cause DNA-crosslinking (Ayala et al., 2014). High MDA expression in brain is associated with release of pro-inflammatory cytokines (e.g. TNF-*a*, IL-1, IL-1*β*, IL-6) by microglia. GSH and catalase are the antioxidant guards of brain. GSH is a tripeptide that forms endogenous free radical scavenger of brain. Decrease in GSH content predisposes the brain towards oxidative stress mediated neurodegeneration. Fortification of GSH content in brain has been utilized as a therapeutic strategy to combat many neurodegenerative disorders such as Alzheimer's disease, PD and Huntington's disease.

In the present study administration of EEA (250 and 500 mg/kg) attenuated the rise of brain TBARS level and decline of the GSH content and catalase activity in intranigral rotenone-treated rats. The potent antioxidant activity of EEA can be attributed to diverse compounds such as hexadecanoic acid methyl ester, squalene, and several phenolic compounds (e.g. caffeic acid, ferulic acid, rosmarinic acid) that have depicted reduction in free radicals toxicity in different animal studies (Ojha et al., 2015; Das et al., 2017). These findings are in harmony with previous results that suggested the antioxidant potential of EEA (Prabakaran et al., 2018). Furthermore, administration of PPX abrogated the ROT triggered oxidative stress in the brain of rats. Therefore, the present study suggests that amelioration of intragiral-rotenone induced oxidative-toxicity by EEA in brain of rats may prove a useful therapeutic intervention in pathology of Parkinson's disease.

CONCLUSIONS

The results of the study demonstrated that ethanol extract of *Epipremnum aureum* leaves bestow neuroprotective effect against rotenone triggered oxidative stress and thereby alleviate the motor-symptoms such as hypokinesia, rigidity and postural instability in Parkinson's disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Sood S	Kumar M	Bansal N
Concepts or ideas	x		x
Design	x	x	x
Definition of intellectual content			x
Literature search	x	x	x
Experimental studies	x	x	x
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Statistical analysis	x	x	x
Manuscript preparation	x	x	x
Manuscript editing		x	x
Manuscript review	x	x	x

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