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Research Article

Evaluation of anti-parkinsonian activity of *Elaeocarpus ganitrus* on haloperidol induced Parkinson's disease in mice

Harish G. Bagewadi*, A. K. Afzal Khan

Department of Pharmacology, MVJ Medical College & Research Hospital, Bengaluru, Karnataka, India

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***Correspondence to:**
Harish G. Bagewadi,
Email: harish2957@gmail.com

ABSTRACT

Background: *Elaeocarpus ganitrus* (Family: Elaeocarpaceae), has been used for the treatment of depression, convulsions and asthma. The existing literature is lacking in studies showing anti-parkinson effect of *E. ganitrus.* There is increased concern about the side-effects of conventional medicine in the treatment of Parkinson's disease (PD). Hence *E. ganitrus* having anti-oxidative property may be a safer alternative.
Methods: To evaluate the anti-parkinson effect of *E. ganitrus*, rota rod and catalepsy bar tests were used. Assessment of oxidative stress was done by measuring the malondialdehyde (MDA) and reduced glutathione (GSH) levels in the striatal region of the brain. One-way ANOVA was used to detect statistical significance, followed by *post-hoc* Tukey test.
Results: *E. ganitrus* (200 and 400 mg/kg, p.o.) pretreated groups significantly increased the retention time in rota rod test ($p<0.001$) and significantly decreased the latency period in catalepsy bar test ($p<0.001$), when compared with haloperidol treated group alone. *E. ganitrus* (200 and 400 mg/kg, p.o.) pretreated groups showed significant anti-oxidative effect by causing a decrease in brain MDA levels ($p<0.001$) and a significant increase in GSH levels ($p<0.001$).
Conclusions: Oxidative stress plays a vital role in the pathophysiology of PD. The results of this study conclusively show that *E. ganitrus* has anti-oxidant activity and neuroprotective activity in haloperidol experimental model of PD.

Keywords: *Elaeocarpus ganitrus*, Anti-oxidant, Haloperidol, Malondialdehyde, Glutathione

INTRODUCTION

Since ancient times, plants have been an exemplary source of medicine to treat various diseases.[1] Decoctions made from fruits of *Elaeocarpus ganitrus* is used in the treatment of epilepsy, asthma, liver disorder, dropsy and hypertension.[2,3] It is also reported to exhibit various pharmacological activities including analgesic[4] and smooth muscle relaxant[5] effects.

The clinical syndrome of PD results from idiopathic degeneration of the dopaminergic cells in the pars compacta of the substantia nigra.[6] Among the causes of the degenerative process, oxidative stress is said to play an integral part.[7]

Among the available pharmacological treatments, levodopa remains the most efficacious and is still the mainstay of therapy. However, long-term use of levodopa leads to disabling motor complications, particularly dyskinesias and motor fluctuations, which limit its further usage. Because of the concern about the side-effects of conventional medicine, there is serious consideration and search for the use of natural products as an alternative to conventional treatment. These natural sources having anti-oxidant and neuroprotective actions can be a good alternative in improving the treatment of Parkinson's disease (PD). Existing literature is lacking in studies showing anti-parkinsons effect of *E. ganitrus.* Hence, efforts have been made in the present study to explore the effects of *E. ganitrus* on the animal model of PD by investigating its effect on behavioral models and oxidative stress changes induced by haloperidol in mice.

METHODS

Swiss albino mice of either sex weighing between 25 and 30 g, obtained from the Central Animal House of University College of Medical Sciences and Guru Teg Bahadur Hospital. The animals were housed in

polypropylene cages in groups of six to eight mice per cage and were maintained under controlled environmental condition (temperature 22±2°C, humidity 50-55%, natural light/day cycle). All the experiments were performed at daytime between 09:30 and 15:30 hrs. Care of animals was according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forest, Government of India, New Delhi. Permission was taken by Institutional Animal Ethics Committee, University College of Medical Sciences, Delhi, (Approval No. IAEC/2011/49 dated 10 March 2011) to carry out the study.

Plant material

E. ganitrus extract was obtained from M/s Tapovan ayurved sadan, New Delhi. As per the literature given by the manufacturer, the dried fruits of *E. ganitrus*, Roxb were powdered initially. 200 g of the drug was soaked in 90% ethanol for 48 hrs. Later, percolation was done through suction by Sauxlet apparatus. Filtration was repeated through Whatman filter paper No. 4 and the filtrate was air dried. The dried extracts were stored at 4°C until further use. The yield of the drug was 05.72% (w/w in terms of dried starting material.) For the purpose of the study, the *E. ganitrus* powder was dissolved in 0.5% carboxy methyl cellulose as a vehicle to prepare suspensions of required doses of 100, 200 and 400 mg/kg.

Experimental design

The animals were divided into six groups (n=12).

Group I - was administered 0.5% carboxy methyl cellulose (orally, once/day ×1 week).
Group II - received haloperidol (1 mg/kg, i.p. once/day ×1 week).
Group III, IV and V - were administered with *E. ganitrus* (100, 200, and 400 mg/kg/day) orally, respectively, ×1 weeks along with haloperidol.
Group VI - received levodopa (30 mg/kg, i.p. once/day ×1 week) along with haloperidol.

E. ganitrus (100 mg/kg, 200 mg/kg, 400 mg/kg) orally and levodopa (30 mg/kg, i.p.) were administered 30 mins prior to injection of haloperidol for 7 days of experimental period. Haloperidol, levodopa were obtained from Sigma Chemical Co. USA and all other chemicals used were of analytical grade.

Assessment of behavioral tests

1. Rota rod test
   The rota rod method used was similar to the one described by Dunham and Miya.[8] The speed selector was set so that the roller rod would make 15 rpm. Before the test, each animal was given 1 min exposure to the moving rod. The animals were placed on the roller for 3 mins. Latency to fall from rolling rod was noted. A normal animal could maintain its equilibrium for an indefinite period. Movement impairment was indicated by the inability of the animal to remain on the roller for a 3 mins test period.
2. Catalepsy bar test
   The test was performed by the method as described by Hoffman and Donovan.[9] Catalepsy was measured by means of a standard bar test, as the time during which the animal maintained an imposed position with both front limbs raised and resting on wooden bar (diameter, 0.7 cm) 9 cm above the surface. The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. Catalepsy was induced with haloperidol. Latency period at different time point intervals (0, 60, 120, 180, 240 mins) after haloperidol administration were added and expressed as average latency period. A cut off time of 180 sec was applied.

At the end of 7 days of the experimental period, the animals were sacrificed using ether anesthesia and brains were taken out for assessment of oxidative stress changes.

Assessment of oxidative stress

Assessment of oxidative stress was done in the striatal region of the brain by malondialdehyde (MDA) and reduced glutathione (GSH) estimation.

Estimation of MDA

MDA (indicator of lipid peroxidation) was estimated as described by Ohkawa et al.[10] Thiobarbituric acid was added to the brain homogenate under acidic conditions and the absorbance of color that developed after heating was estimated spectrophotometrically at 535 nm.

Estimation of reduced GSH

Reduced GSH was estimated by the method described by Ellman.[11] This method is based on the development of a yellow color when 5,5'-dithio-bis-2-nitrobenzoic acid is added to compounds containing sulfhydryl groups.

Statistical analysis

Results of the above experiments were expressed as mean±standard error mean, and the difference between means was analyzed by analysis of variance using Graph Pad Prism, followed by *post-hoc* Tukey test, with $p<0.05$ being considered as statistical significant.

RESULTS

Rota rod test

In the group, which received only haloperidol, a significant decrease in retention time (p<0.001) was seen on day "0" and day "07" when compared to the control group. In levodopa treated group, a significant increase in retention time (p<0.001) was seen on the day "0" and the day "07" when compared to haloperidol treated group. However, unlike levodopa treated group, *E. ganitrus* 100 mg/kg, 200 mg/kg and 400 mg/kg pretreated groups did not cause any significant change in retention time on the day "0." However, on day "07" *E. ganitrus* 200 mg/kg and 400 mg/kg groups showed significant increase in retention time (p<0.001) when compared to haloperidol treated group as shown in Table 1. Whereas, no significant difference in retention time was seen when *E. ganitrus,* 400 mg/kg treated group was compared with levodopa treated group.

Catalepsy bar test

In the group which received only haloperidol, significant increase in latency period (p<0.001) was seen on day "0" and the day "07" as compared to the control group. In levodopa treated group, a significant decrease in the latency period (p<0.001) on day "0" and the day "07" was seen as compared to haloperidol treated group. However, unlike levodopa treated group, *E. ganitrus* 100 mg/kg, 200 mg/kg and 400 mg/kg pretreated groups did not cause any significant change in the latency period on the day "0." However, on day "07" *E. ganitrus* 200 mg/kg and 400 mg/kg groups showed significant decrease in latency period (p<0.001) when compared to haloperidol treated group as shown in Table 2. Whereas, no significant difference in latency period was seen when *E. ganitrus* 400 mg/kg treated group compared to levodopa treated group.

Estimation of MDA

In the haloperidol treated group, a significant increase in brain MDA levels (p<0.001) was seen as compared to the control group. *E. ganitrus* 200 mg/kg, 400 mg/kg and levodopa pretreated groups showed significant decrease (p<0.001) in brain MDA levels when compared to haloperidol treated group as shown in Table 3. *E. ganitrus* 200 and 400 mg/kg treated groups did not show significant difference in brain MDA levels when compared to levodopa treated group.

Estimation of reduced GSH

In the haloperidol treated group, a significant decrease in brain GSH levels (p<0.001), was seen as compared to the control group. *E. ganitrus* 200 mg/kg, 400 mg/kg and levodopa pretreated groups showed significant increase (p<0.001) in brain GSH levels when compared to haloperidol treated group as shown in Table 4. *E. ganitrus* 200 and 400 mg/kg treated groups did not show significant difference in brain GSH levels when compared to levodopa treated group.

DISCUSSION

PD is a commonly diagnosed neurodegenerative disorder, characterized by degeneration of dopamine producing

Table 1: Effect of *E. ganitrus* on rota rod test in haloperidol treated mice.

Groups, (dose)	Retention time (sec) 0 day	Retention time (sec) 7th day
CMC (1 ml/kg, p.o)	90.8±4.06	168.2±2.5
HAL (1 mg/kg, i.p.)	30.2±4.18*	21.6±3.21*
E. ganitrus (100 mg/kg, i.p.)+HAL	31±4.18*,‡	50.7±3.21*,‡
E. ganitrus (200 mg/kg, i.p.)+HAL	31.8±4.15*,†,‡	115.7±4.34*,†,‡
E. ganitrus (400 mg/kg, i.p.)+HAL	40.1±4.45*,†,‡	141.5±5.30*,†
Levodopa (30 mg/kg, i.p.)+HAL	67.8±3.19*,†	159.4±3.69†

n=12, values expressed as mean±SEM. *p<0.001 versus CMC-control, †p<0.001 versus HAL, ‡p<0.05 versus (levodopa+HAL), CMC: Carboxy methyl cellulose, HAL: Haloperidol, SEM: Standard error mean, *E. ganitrus: Elaeocarpus ganitrus*

Table 2: Effect of *E. ganitrus* on catatonic response in haloperidol treated mice.

Groups, (dose)	Latency period (sec) 0 day	Latency period (sec) 7th day
CMC (1 ml/kg, p.o)	18.6±2.31	31.2±3.33
HAL (1 mg/kg, i.p.)	161.2±3.24*	213.5±2.57*
E. ganitrus (100 mg/kg, i.p.)+HAL	157±4.18*,‡	145.7±3.21*,‡
E. ganitrus (200 mg/kg, i.p.)+HAL	155.2±4.32*,†,‡	73.2±5.44*,†,‡
E. ganitrus (400 mg/kg, i.p.)+HAL	152.9±4.54*,†,‡	53.8±5.27*,†
Levodopa (30 mg/kg, i.p.)+HAL	95.2±3.30*,†	36.6±2.20†

n=12, values expressed as mean±SEM. *p<0.001 versus CMC, †p<0.001 versus HAL, ‡p<0.05 versus (levodopa+HAL), CMC: Carboxy methyl cellulose, HAL: Haloperidol, SEM: Standard error mean, *E. ganitrus: Elaeocarpus ganitrus*

Table 3: Effect of *E. ganitrus* on brain levels of MDA in haloperidol treated mice.

Groups, (dose)	MDA (nmol/g tissue)
CMC (1 ml/kg, p.o)	175.1±3.38
HAL (1 mg/kg, i.p.)	520.6±7.47*
E. ganitrus (100 mg/kg, i.p.)+HAL	413.3±8.27*
E. ganitrus (200 mg/kg, i.p.)+HAL	262.7±8.69*,†
E. ganitrus (400 mg/kg, i.p.)+HAL	240.2±8.07*,†
Levodopa (30 mg/kg, i.p.)+HAL	223.3±9.43†

n=12, values expressed as mean±SEM. *p<0.001 versus CMC, †p<0.001 versus HAL. HAL: Haloperidol, SEM: Standard error mean, *E. ganitrus: Elaeocarpus ganitrus*, MDA: Malondialdehyde, CMC: Carboxy methyl cellulose

Table 4: Effect of *E. ganitrus* on brain levels of GSH in haloperidol treated mice.

Groups, (Dose)	GSH (µg/g tissue)
CMC (1 ml/kg, p.o)	457.8±9.03
HAL (1 mg/kg, i.p.)	127.2±7.12*
E. ganitrus (100 mg/kg, i.p.)+HAL	520.3±8.27*
E. ganitrus (200 mg/kg, i.p.)+HAL	406.5±7.15*,†
E. ganitrus (400 mg/kg, i.p.)+HAL	429.6±5.25*,†
Levodopa (30 mg/kg, i.p.)+HAL	447.7±4.27†

n=12, values expressed as mean±SEM. *p<0.001 versus CMC, †p<0.001 versus HAL, HAL: Haloperidol, SEM: Standard error mean, *E. ganitrus: Elaeocarpus ganitrus*, MDA: Malondialdehyde, CMC: Carboxy methyl cellulose, GSH: Glutathione

neurons in the substantia nigra leading to resting tremor, bradykinesia, shuffling gait, flexed posture and rigidity.

Still, the cause of the degeneration is not well defined. Oxidative stress may play a major role.[7] Oxidative stress may arise from the metabolism of dopamine with the generation of harmful free radicals.[12] Compared to the rest of the brain, the substantia nigra pars compacta is exposed to a higher rate of free radical formation and to increased level of oxidative stress. This may be related to the energy metabolism of these cells or to their more content of dopamine.[13] Various studies have revealed oxidative stress changes evident in the brain of PD patients.[14]

Haloperidol, a neuroleptic drug, induces catalepsy which is due to a blocking of post synaptic striatal dopamine D2 receptors and many studies have shown reactive oxygen species as a cause of haloperidol induced toxicity.[15] Drugs which attenuate haloperidol-induced motor disorders might reduce the extrapyramidal signs of PD.

In our study, two behavioral parameters - rota rod performance and a catatonic response were measured as retention time (sec) and the latency period (sec), respectively. In Haloperidol treated group, 07 days treatment with *E. ganitrus* (200, 400 mg/kg, p.o.), significantly increased the retention time (sec) in rota rod test and decreased the latency period (sec) in catalepsy bar test and this effect was comparable to that of levodopa group. The findings of behavioral tests of our study are similar to other studies conducted previously.[16]

The assessment of biochemical parameters of oxidative stress was done by measuring brain MDA and reduced GSH levels. Haloperidol treated group showed significant increase in brain MDA and decrease in GSH levels. *E. ganitrus* (200, 400 mg/kg) and levodopa caused significant decrease in brain MDA and increase in brain GSH levels. The results of biochemical tests of our study are in accordance with previous studies as well.[15] Thus, the oxidative stress parameters (MDA and GSH) are also positively modulated by *E. ganitrus* so as to decrease the oxidative damage to neurons.

E. ganitrus is an important medicinal plant that plays a significant role in protection from oxidative stress. It has been hypothesized that anti-oxidants may be neuroprotective in PD, by preventing neuronal degeneration caused by intracellular free radicals.[7]

It is widely accepted that inflammation and oxidative stress are interrelated. Oxidative stress can increase inflammatory activity and conversely, inflammation is known to cause oxidative stress.[17] The role of neuroinflammation in PD have coincided with increasing interests in determining whether anti-inflammatory medications might be helpful in preventing PD. Recently, involvement of inflammatory process has also been reported in the pathogenesis of PD.[13] Experimental evidence on animal models support a preventative role for non-steroidal anti-inflammatory drugs in PD.[18]

The petroleum ether, benzene, chloroform, acetone and ethanol extracts of fruits of *Euchiton sphaericus* exhibited anti-inflammatory activities in various experimental models of inflammation in rats.[19] Phytochemical screening of ethanolic extract of fruits shows the presence of alkaloids, flavonoids, carbohydrates, proteins and tannins.[20] The ethanolic extract of leaves of *E. ganitrus* yielded quercetin, gallic and ellagic acids, (±) elaeocarpine, (±) iso-elaeocarpine.[21] Alkaloids (elaeocarpidine, elaeocarpine[22] and rudrakine[23]) are reported to be the major phytoconstituents of *E. sphaericus.*

Flavonoids like quercetin,[24] phenolics are also reported to be the phytoconstituents of *E. sphaericus*. Lower levels of lipid peroxides in the brains of the drug treated group and increased activities of enzymatic and non-enzymatic anti-oxidants in the brain suggest that the extract decreases the oxidative stress damage.[25] Flavonoids from *E. ganitrus* leaves have substantial anti-oxidant activity.[26] The anti-

parkinsonian effect of *E. ganitrus* may be attributed to its anti-oxidant action due to the presence of alkaloids and flavonoids.

The present investigation shows that *E. ganitrus* fruit has anti-parkinson activity in haloperidol induced PD in mice. Whether *E. ganitrus* has anti-oxidant and neuroprotective activity in other experimentally induced Parkinsonism models like reserpine, 6-hydroxy dopamine needs further evaluation. Further studies may also be undertaken to identify and investigate the mechanism of action of active anti-parkinsonian compounds in *E. ganitrus.*

CONCLUSION

PD is a progressive neurodegenerative disorder accompanied by loss of dopaminergic neurons of the substantia nigra pars compacta. Haloperidol is commonly used to create experimental model of PD. Haloperidol toxicity leads to generation of free radicals leading to oxidative stress.

The results of the present study conclusively showed that *E. ganitrus* has anti-oxidant activity and neuroprotective role in haloperidol experimental model of PD. *E. ganitrus* was also found to be effective in increasing rota rod performance and decreasing catatonic response. Hence, the neuromodulatory effect of *E. ganitrus* on behavioral, oxidative stress may be due to its neuroprotective and anti-oxidant properties. In this regard, future clinical studies can be undertaken which may provide a ray of hope to use *E. ganitrus* in the treatment of PD.

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