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Beneficial effect of *Elaeocarpus ganitrus* in Haloperidol induced parkinsonian disease in animal model

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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 antiparkinson 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 antioxidative property may be a safer alternative.
Materials & Methods: To evaluate the antiparkinson 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 to 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 vital role in pathophysiology of Parkinson's disease. The results of the present study conclusively show that *E.ganitrus* has antioxidant activity and neuroprotective activity in Haloperidol experimental model of Parkinson's disease.

Keywords: *E.ganitrus*, Anti-oxidant, Haloperidol, MDA, GSH

Introduction
Since ancient times, plants have been an exemplary source of medicine to treat various diseases [1]. Decoctions made from fruits of *E.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 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 antioxidant and neuroprotective actions can be a good alternative in improving the treatment of Parkinson's disease. Existing literature is lacking in studies showing antiparkinsons effect of *E.ganitrus.* So efforts have been made in the present study to explore the effects of *E.ganitrus* on animal model of Parkinson's disease by investigating its effect on behavioral models and oxidative stress changes induced by Haloperidol in mice.

Materials & Methods
Swiss albino mice of either sex weighing between 25 and 30 gms, were obtained. 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 hours. 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 to carry out the study. The study was conducted at Department of Industrial Biotechnology, National Institute of Technology Karnataka, Surathkal, from March 2008 to July 2009. *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 gms 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^{o}C until further use. The yield of the drug was 05.72% (w/w in terms of dried starting material.) For the purpose of study, the *E.ganitrus* powder was dissolved in 0.5% Carboxy methyl cellulose as a vehicle to prepare suspensions of required doses of 200 and 400 mg/kg.

Experimental Design

The animals were divided into 05 groups (*n* =12).

- **Group I**- was administered 0.5% Carboxy methyl cellulose (orally, once per day ×15 days).
- **Group II**- received Haloperidol (1mg/kg, i.p. once per day x 15 days).
- **Group III, IV -** were administered with *E.ganitrus* (200, and 400 mg/kg/day) orally, respectively, ×15 days along with Haloperidol.
- **Group V**- received Levodopa (30mg/kg, i.p. once per day x 15 days) along with Haloperidol.

E.ganitrus (200mg/kg, 400mg/kg) orally and Levodopa (30mg/kg, i.p.) were administered 30 minutes prior to injection of Haloperidol for 15 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

i) 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 minute exposure to the moving rod. The animals were placed on the roller for 3 minutes. Latency to fall from rolling rod was noted. A normal animal could maintain its equilibrium for an indefinite period of time. Movement impairment was indicated by the inability of the animal to remain on the roller for a 3 minute test period.

ii) Catalepsy bar test

The test was performed by the method as described by Hoffman *et al*. [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 minutes) after Haloperidol administration were added and expressed as average latency period. A cut off time of 180 seconds was applied.

At the end of 15 days of 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 Malondialdehyde (MDA)

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

Estimation of Reduced Glutathione (GSH)

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

Statistical Analysis

Results of the above experiments were expressed as Mean ± SEM, and the difference between means was analyzed by analysis of variance (ANOVA) 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, significant decrease in retention time ($p<0.001$) was seen on 7th day and 15th day as compared to control group. In Levodopa treated group, significant increase in retention time ($p<0.001$) was seen on7th day and 15th day as compared to Haloperidol treated group. However, unlike Levodopa treated group, *E.ganitrus* 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in retention time on 7th day. But on 15th day *E.ganitrus* 200 mg/kg and 400mg/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* 400mg/kg treated group was compared to Levodopa treated group.

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

Groups, (Dose)	Retention time (Sec) 7th day	Retention time (Sec) 15th day
1. CMC (1ml/kg, p.o)	93.8±2.12	171.2±5.12
2. HAL (1mg/kg, i.p.)	33.2±3.27*	24.6±2.35*
4. *E.ganitrus* (200mg/kg, i.p.) + HAL	34.8±2.17*, †, ‡	118.7±2.31*, †, ‡
5. *E.ganitrus* (400mg/kg, i.p.) + HAL	43.1±2.25*, †, ‡	144.5±3.38*, †
6. Levodopa (30mg/kg, i.p.) + HAL	70.8±2.29*, †	162.4±2.63†

(n=12), values expressed as mean±SEM.

*p< 0.001 vs. Carboxy methyl cellulose (CMC)-control,

†p< 0.001 vs. Haloperidol (HAL), ‡p< 0.05 vs. (Levodopa+ HAL)

Catalepsy bar test

In the group which received only Haloperidol, significant increase in latency period ($p<0.001$) was seen on 7th day and 15th day as compared to control group. In Levodopa treated group, significant decrease in latency period ($p<0.001$) on7th day and 15th day was seen as compared to Haloperidol treated group. However, unlike Levodopa treated group, *E.ganitrus* 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in latency period on 7th day. But on 15th day *E.ganitrus* 200mg/kg and 400mg/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* 400mg/kg treated group compared to Levodopa treated group.

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

Groups, (Dose)	Latency period (Sec) 7th day	Latency period (Sec) 15th day
1. CMC (1ml/kg, p.o)	22.6±3.32	35.2±2.31
2. HAL (1mg/kg, i.p.)	165.2±2.26*	217.5±2.52*
4. *E.ganitrus* (200mg/kg, i.p.) + HAL	159.2±2.31*, †, ‡	77.2±3.46*, †, ‡
5. *E.ganitrus* (400mg/kg, i.p.) + HAL	156.9±2.52*, †, ‡	57.8±3.24*, †
6. Levodopa (30mg/kg, i.p.) + HAL	99.2±1.36*, †	40.6±3.22†

(n=12), values expressed as mean± SEM. *p< 0.001 vs. (CMC), †p< 0.001 vs. Haloperidol (HAL), ‡p< 0.05 vs. (Levodopa+ HAL)

Estimation of Malondialdehyde (MDA)

In the Haloperidol treated group, significant increase in brain MDA levels (p<0.001) was seen as compared to control group. *E.ganitrus* 200mg/kg, 400mg/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 400mg/kg treated groups did not show significant difference in brain MDA levels when compared to Levodopa treated group.

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

Groups, (Dose)	MDA (nmol/g tissue)
1. CMC (1ml/kg, p.o)	180.1±2.31
2. HAL (1mg/kg, i.p.)	525.6±3.45*
4. *E.ganitrus* (200mg/kg, i.p.) + HAL	267.7±4.64*, †
5. *E.ganitrus* (400mg/kg, i.p.) + HAL	245.2±2.02*, †
6. Levodopa (30mg/kg, i.p.) + HAL	228.3±7.42†

(n=12), values expressed as mean±SEM. *p< 0.001 vs. (CMC), †p< 0.001 vs. Haloperidol (HAL)

Estimation of reduced Glutathione

In the Haloperidol treated group, significant decrease in brain GSH levels (p<0.001), was seen as compared to control group. *E.ganitrus* 200mg/kg, 400mg/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 400mg/kg treated groups did not show significant difference in brain GSH levels when compared to Levodopa treated group.

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

Groups, (Dose)	GSH (µg/g tissue)
1. CMC (1ml/kg, p.o)	464.8±9.01
2. HAL (1mg/kg, i.p.)	134.2±7.13*
4. *E.ganitrus* (200mg/kg, i.p.) + HAL	413.5±7.17*, †
5. *E.ganitrus* (400mg/kg, i.p.) + HAL	435.6±5.23*, †
6. Levodopa (30mg/kg, i.p.) + HAL	454.7±4.22†

(n=12), values expressed as mean±SEM. *p< 0.001 vs. (CMC), †p< 0.001 vs. Haloperidol (HAL)

Discussion

Parkinson's disease is a commonly diagnosed neurodegenerative disorder, characterized by degeneration of dopamine producing 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 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 are evident in the brain of Parkinson's disease 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 Parkinson's disease.

In our study, two behavioral parameters - rota rod performance and catatonic response were measured as retention time (sec) and latency period (sec) respectively.

In Haloperidol treated group, 15 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 malondialdehyde (MDA) and reduced glutathione (GSH) levels. Haloperidol treated group showed significant increase in brain MDA and decrease in GSH levels. *E.ganitrus* (200, 400mg/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 antioxidants 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 Parkinson's disease 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 Parkinson's disease [13]. Experimental evidence on animal models support a preventative role for nonsteroidal anti-inflammatory drugs (NSAIDs) in Parkinson's disease [18].

The petroleum ether, benzene, chloroform, acetone and ethanol extracts of fruits of *E.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

and rudrakine) [22, 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 nonenzymatic antioxidants in the brain suggest that the extract decreases the oxidative stress damage [25]. Flavonoids from *E.ganitrus* leaves have substantial antioxidant activity [26]. The antiparkinsonian effect of *E.ganitrus* may be attributed to its antioxidant action due to the presence of alkaloids and flavonoids.

The present investigation shows that *E.ganitrus* fruit has antiparkinson activity in Haloperidol induced parkinson disease in mice. Whether *E.ganitrus* has antioxidant and neuroprotective activity in other experimentally induced Parkinsonism models like Reserpine, 6-OHDA (6-Hydroxy dopamine) needs further evaluation. Further studies may also be undertaken to identify and investigate the mechanism of action of active antiparkinsonian compounds in *E.ganitrus.*

Conclusion

Parkinson's disease 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 Parkinson's disease. Haloperidol toxicity leads to generation of free radicals leading to oxidative stress. The results of the present study conclusively showed that *E.ganitrus* has antioxidant activity and neuroprotective role in Haloperidol experimental model of Parkinson's disease. *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 antioxidant 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 Parkinson's disease.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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