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Beneficial effect of *Elaeocarpus ganitrus* on MPTP induced parkinson's disease in mice

Harish .G. Bagewadi*[1], Naresh Khanna[2], Chetan .S. Bhangale[3] and Proteesh Rana[4]

[*1]Asst.Professor, Department of Pharmacology, MVJ Medical College & Research Hospital, Bangalore, India-562114

[2]Professor, [3]Senior Resident, Department of Pharmacology, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi, India-110095

[4]Senior Resident- Department of Pharmacology, Maulana Azad Medical College, New Delhi, India-110095

***Corresponding author: Harish .G. Bagewadi**

E-mail id: harish2957@gmail.com

ABSTRACT

Aim of the study: *Elaeocarpus ganitrus* (Family: Elaeocarpaceae), has shown benefit in the treatment of depression, convulsions and asthma. This study was undertaken to evaluate the antiparkinson effect of *E.ganitrus*.

Materials and methods: Parkinson's disease was induced by administering MPTP (2 doses, each dose 20 mg/kg at 2 hr. interval, i.p. daily x 1week).The mice of either sex were divided into 06 groups (n =12). 1st group mice were given 0.5% Carboxy methyl cellulose (orally), 2nd group were administered MPTP (2 doses, each dose 20 mg/kg at 2 hr. interval, i.p.). Whereas 3rd, 4th and 5th groups - were administered with *E.ganitrus* (100, 200, and 400 mg/kg/day,orally), respectively, along with MPTP. Group 6- received Levodopa (30mg/kg, i.p,) along with MPTP. Retention time in Rota rod test and latency period in catalepsy bar test was measured on day 0 and day 7. Assessment of oxidative stress was done by malondialdehyde (MDA) and reduced glutathione (GSH) measurement. One way ANOVA followed by post-hoc Tukey test, with $p<0.05$ considered statistical significant.

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 MPTP treated group alone. *E.ganitrus* (200 and 400 mg/kg, p.o.) pretreated groups showed significant antioxidative effect by decrease in brain MDA levels ($p <0.001$) and significant increase in brain GSH levels ($p <0.001$).

Conclusions: In our study, pretreatment with *E. ganitrus* showed antioxidative activity and increase in retention time in Rota rod test and decrease in latency period in Catalepsy bar test. The anti-oxidative property might be responsible for the changes in behavioral test parameters in the MPTP induced Parkinson's disease in mice.

Keywords: *E.ganitrus*, antioxidant, MPTP, MDA, GSH

INTRODUCTION

Plants are exemplary source of medicine over the years to treat many diseases [1]. Decoctions prepared from fruits of *Elaeocarpus Ganitrus* (*E.ganitrus)* have showed better results in the treatment of epilepsy, asthma, liver disorder, dropsy and hypertension [2, 3]. In addition to these benefits, *E.ganitrus* has found to exhibit many pharmacological activities that include analgesic [4], smooth muscle relaxant effects [5]. Parkinson's disease is a progressive neuro degenerative disorder which mainly results from idiopathic degeneration of dopaminergic cells in the substantial nigra pars compact [6]. While the causative factors for the degeneration of dopaminergic cells in the substantial nigra pars compact is still not well-established, oxidative stress might play an important role [7]. Levodopa remains the most efficacious drug in pharmacotherapy of Parkinson's disease. However, long-term use of levodopa causes disabling motor complications like dyskinesia's and on-off phenomenon. Because of the concern over the side effects of allopathic medicines, the use of natural products which may serve as better alternative to existing treatment needs to be explored. Thus; strategies employing antioxidant activity with lesser side effects from natural sources can be a good approach in improving the treatment of Parkinson's disease. The lacunae in existing literature lack 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, oxidative stress changes induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydro pyridine (MPTP) in mice.

MATERIALS AND METHODS

Swiss albino mice of either sex weighing between 25 and 30 g were obtained from the central animal house of University College of Medical Sciences and Guru Tag Bahadur Hospital. The mice were housed in cages and kept under controlled environmental condition (temperature 22±2 °C, humidity 50–55 %, natural light/day cycle). All the experiments were performed in daytime between 09:30 and 15:30 hours. Care of animals was given according to the CPCSEA guidelines. Permission was taken from the Institutional Animal Ethics Committee to carry out the study (Approval No. IAEC/2011/49 dated 10 March 2011).

Plant extract and Chemicals

E.ganitrus extract was obtained from M/s Tapovan ayurved sadan, New Delhi. As per the literature provided by the manufacturer, initially the dried fruits of *E.ganitrus*, Roxb were taken and crushed into a fine powder. 200 Gms of the drug was soaked in 90% ethanol for 48 hrs. Percolation was carried out by Soxhlet apparatus through suction method. Filtration was per formed repeatedly with the help of what man filter paper No-4 and filtrate was air dried sufficiently. Then the dried extracts were stored at 4^{o}C until its further usage. The yield of the drug was found to be 05.72% (w/w in terms of dried starting material). To carry out our study, the *E. ganitrus* powder was dissolved in 0.5% Carboxy methyl cellulose (CMC) to prepare suspensions of different doses of 100, 200 and 400 mg/kg. MPTP and Levodopa was obtained from Sigma Chemical Co. USA. The different doses of ethanolic extract of *E.ganitrus* were chosen from other previous studies conducted by Kakalij RM et al [8], and Bagewadi HG et al [9].

Experimental Design

Swiss albino mice (6 weeks old) of either sex weighing between 25 and 30 Gms were used for the study. The mice were divided into 06 groups (n =12).Group I- was administered 0.5% Carboxy methyl cellulose (orally ×1 weeks). Group II- received MPTP (2 doses, each dose 20 mg/kg at 2 hr. interval, i.p. daily x 1 week). Groups III, IV, and V- were treated with *E.ganitrus* (100, 200, and 400 mg/kg/day, orally), respectively for 1 week along with MPTP. Group VI- received Levodopa (30mg/kg, i.p, once per day x 1 week) along with MPTP. The *E.ganitrus* (100mg/kg, 200mg/kg, 400mg/kg, orally) and Levodopa (30mg/kg, i.p.) were given 30 minutes prior to injections of first dose of MPTP for 7 days of experimental period. The behavioral parameters were assessed on day 0 and day 07. MPTP, Levodopa was obtained from Sigma Chemical Co. USA.

ASSESSMENT OF BEHAVIORAL TESTS

1. Rota rod test

This test was performed by the method one described by Dunham and Miya [10]. The speed selector was adjusted such that roller rod would make 15 revolutions per minute. Prior to the test; each mouse was given 1 minute exposure to the moving rod for its acclimatization. The mice were kept for 3 minutes test period and latency to fall from moving rod was noted. The inability of the mice to maintain balance on the moving rod for 3 minute test period indicated the movement impairment in mice. On day 0 and day 07, rotarod test was performed after one hour of last dose administration.

2. Catalepsy bar test

This test was performed by the method as described by Hoffman et al [11]. Catalepsy was assessed by means of a standard bar test on the wooden bar having diameter- 0.7 cm and height- 9 cm. The mouse would remain in an imposed position with both front limbs raised and resting on the wooden bar. The mouse when it removed either front paws from the bar or when mouse moved its head in an exploratory manner it indicated the occurrence of end point of catalepsy. After MPTP administration, the latency period at 60 minutes intervals (0, 60, 120, 180, 240 minutes) were recorded and expressed as average latency period. A cut off time of 180 seconds was applied. On day 0 and day 07, catalepsy bar test was performed after one hour of last dose administration.

ASSESSMENT OF OXIDATIVE STRESS

At the end of 7 days of experimental period, mice were administered high dose of (Pentobarbitone sodium) anesthesia and brains were taken out for assessment of oxidative stress changes. Assessment of oxidative stress was done in the striate region of the mice brain by malondialdehyde (MDA) and reduced glutathione (GSH) in each group.

Estimation of Malondialdehyde (MDA)

Malondialdehyde (which is an indicator of lipid per oxidation) was estimated as described by Okhawa et al [12]. Thiobarbituric acid was added to the brain homogenate under acidic conditions. The absorbance of color that developed after heating the brain homogenate was recorded with the help of spectrophotometer at 535 nm.

Estimation of Reduced Glutathione (GSH)

Reduced glutathione was estimated by the method described by Ellman [13].This method is based on the appearance of a yellow color when 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB) is added to sulfhydryl groups containing ingredients.

Statistical Analysis

Results of the above experiments were expressed as Mean ± SEM, and the difference between means was analyzed by one-way analysis of variance (ANOVA) using graph pad followed by post-hoc Tukey's test, with $p<0.05$ was considered as statistical significant.

RESULTS

Rota rod test

It was observed that MPTP alone treated group, significantly decreased the retention time ($p<0.001$) on day 0 and day 07 as compared to control group. In Levodopa treated group, significant increase in retention time ($p<0.001$) was seen on both day 0 and day 7, as compared to MPTP treated group. *E.ganitrus* 100 mg/kg, 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in retention time on day 0. But on day 7, *E.ganitrus* 200 mg/kg and 400mg/kg pretreated groups showed significant increase in retention time ($p<0.001$) when compared to MPTP (as shown in table:1), Whereas no significant difference in retention time was seen when compared to levodopa treated group.

Catalepsy bar test

It was observed that among MPTP alone treated group, significant increase in latency period ($p<0.001$) was seen on day 0 and on day 7 when compared to control group. In Levodopa treated group, significant decrease in latency period ($p<0.001$) on day 0 and day 7 was seen when compared to MPTP treated group. *E.ganitrus* 100 mg/kg, 200mg/kg and 400mg/kg pretreated groups did not cause any significant change in latency period on day 0. But on day 7, *E.ganitrus* 200mg/kg and

400mg/kg pretreated groups showed significant decrease in latency period ($p<0.001$) when compared to MPTP treated group (as shown in table 1), whereas no significant difference in latency period was seen when compared to levodopa treated group.

Estimation of Malondialdehyde (MDA)

In MPTP treated group, significant increase in brain MDA levels ($p<0.001$) was seen when 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 MPTP treated group (as shown in table 2). *E.ganitrus* 200 and 400mg/kg pretreated groups did not show significant difference in brain MDA levels when compared to Levodopa treated group.

Estimation of reduced Glutathione (GSH)

In MPTP treated group, significant decrease in brain GSH levels ($p<0.001$) was seen as compared to control group. *E.ganitrus* 200mg/kg, 400 mg/kg and Levodopa pretreated groups showed significant increase ($p<0.001$) in brain GSH levels when compared to MPTP treated group (as shown in table 2). *E.ganitrus* 200 and 400mg/kg pretreated groups did not show significant difference in brain GSH levels when compared to Levodopa treated group.

Table1. Effect of *E.ganitrus* on Rota test (retention time) and Catalepsy bar test (Latency period) in MPTP treated mice.

Groups, (Dose)	Retention time (Sec) Day 0	Retention time (Sec) Day 7	Latency period (Sec) Day 0	Latency period (Sec) Day 7
1. CMC (1ml/kg, p.o)	72.8±3.76	77.4±2.15	16.4±1.79	30.3±2.13
2. MPTP (20 mg/kg, i.p.)	25.4±2.37*	18.2±2.58*	170.3±3.35*	224.8±5.01*
3.*E.ganitrus* (100mg/kg, i.p.) +MPTP	27.2±4.18*,‡	53.7±3.21*,‡	157±4.18*,‡	145.7±3.21*,‡
4.*E.ganitrus* (200mg/kg, i.p.) +MPTP	29.4±3.10*,†,‡	116.5±4.54*,†,‡	168.3±5.29*,†,‡	72.4±4.25*,†
5.*E.ganitrus* (400mg/kg, i.p.) +MPTP	31.7±3.53*,†,‡	136.5±3.92*,†	166.7±4.82*,†,‡	57.1±5.55*,†
6.Levodopa (30mg/kg, i.p.) +MPTP	70.1±4.53*,†	157.8±3.73*,†	101.2±2.82*,†	40.7±3.19*,†

The results are expressed as mean ± SD for 12 animals in each group. $^{*}p < 0.001$ vs. Carboxy methyl cellulose (CMC) - control, $^{\dagger}p < 0.001$ vs. MPTP, $^{\ddagger}p < 0.001$ vs. (Levodopa + MPTP)

Table2. Effect of *E.ganitrus* on brain levels of MDA and GSH in MPTP treated mice.

Groups, (Dose)	MDA (nmol/g tissue)	GSH (µg/g tissue)
1. CMC (1ml/kg, p.o)	175.1±3.38	457.8±9.03
2. MPTP (20 mg/kg, i.p.)	530.8±6.68*	130.7±5.28*
3. *E.ganitrus* (100mg/kg, i.p.) + MPTP	482.3±6.2*	146.2±4.5*
4. *E.ganitrus* (200mg/kg, i.p.) + MPTP	281.4±10.15*,†	405.7±6.51*,†
5. *E.ganitrus* (400mg/kg, i.p.) + MPTP	265.2±6.83*,†	423.8±8.73*,†
6.Levodopa (30mg/kg, i.p.) + MPTP	245.2±8.31*,†	442.8±7.21*,†

The results are expressed as mean ± SD for 12 animals in each group. $^{*}p < 0.001$ vs. (CMC), $^{\dagger}p < 0.001$ vs. MPTP

DISCUSSION

Parkinson's disease is a progressive neurodegenerative disorder, characterized by degeneration of dopaminergic neurons in the substantial nigra pars compacta leading to resting tremor, bradykinesia, flexed posture, shuffling gait

and rigidity. The cause for dopaminergic neuronal degeneration is still not well established, but oxidative stress might play significant role [7]. Oxidative stress may result from generation of free radical reactive species during the metabolism of dopamine [14].The substantia nigra pars compacta is relatively more succumbed to generation of free radical reactive oxygen species amounting to more oxidative stress. This excess generation of free radical reactive oxygen species (ROS) might be directly correlated to the high energy metabolism or to more content of dopamine in these cells [15]. Several studies have established the finding that oxidative stress changes are demonstrable in the brain of Parkinson's disease patients [16]. (MPTP), 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine is a potent neurotoxin used to create an experimental model of Parkinson's disease in animals. Certain aspects of the Parkinson's disease such as catalepsy, motor incoordination and bradykinesia can be easily studied in this model. As MPTP is highly lipophilic, makes it enable to cross the blood brain barrier immediately after its systemic absorption. Once MPTP reaches the brain tissue, it is converted to the hydrophilic metabolite 1-methyl-4 phenyl pyridinium ion (MPP^+), the free radical reactive specie in the causation of dopaminergic neuronal loss. It is established that these free radical reactive species play a vital role in the pathogenesis of dopaminergic neuronal loss in Parkinson's disease [17]. *E.ganitrus* is an important medicinal plant which has proved its benefits in various neurological diseases caused by oxidative stress. It is convincingly explained that antioxidants might be effective in PD by preventing neuronal death which is caused by intracellular free radicals [7]. The two behavioral parameters - Rota rod performance and catatonic response were measured as retention time (sec) and latency period (sec) respectively. In MPTP treated group, 7 days pretreatment with *E.ganitrus*(200, 400 mg/kg, p.o.), significantly increased the retention time (sec) in Rota rod test, decreased the latency period (sec) in catalepsy bar test and this effect is comparable to that of levodopa group. The above findings of behavioral tests are in accordance with other previous studies [18, 19]. The assessment of biochemical parameters of oxidative stress were done by measuring brain malondialdehyde (MDA) and reduced glutathione (GSH) levels. MPTP treated group showed significant increase in brain MDA and decrease in GSH levels. Whereas, *E.ganitrus* (200, 400mg/kg) and levodopa caused significant decrease in brain MDA and increase in brain GSH levels. Our results of biochemical tests are in accordance with previous studies [20]. The role of neuroinflammation and usage of anti-inflammatory medication in the prevention of Parkinson's disease still needs to be well established. However, some animal experimental studies have showed preventative role of nonsteroidal anti-inflammatory drugs (NSAID's) in Parkinson's disease. Recently, it became evident that inflammatory process contributes significantly to the pathogenesis of Parkinson's disease[15, 21].Previous studies have established the finding that anti-inflammatory drugs such as acetylsalicylic acid can prevent MPTP induced Parkinson's disease in mice [22]. It is widely accepted that inflammation and oxidative stress both are interrelated. It is interesting to know that oxidative stress can potentiate inflammatory process and inflammation is found to generate oxidative stress [22]. Anti-inflammatory activity was demonstrated by the several fruit extracts of *E.sphaericus* like petroleum ether, benzene, Chloroform, acetone and ethanol extracts in rats [23]. The key ingredients identified after phytochemical screening of ethanolic extract of fruits were alkaloids, flavonoids, carbohydrates, proteins and tannins [24]. The ethanol extract of leaves of *E.ganitrus* is also known to contain quercetin, Gallic and pelagic acids (elaeocarpine, isoelaeocarpine) [25]. The major phyto constituents of *E.sphaericus* are mainly alkaloids like-elaeocarpidine, elaeocarpine[26], and rudrakine[27]. Flavonoids like quercetin[28], phenolics are also important phytoconstituents of *E.sphaericus.* Increased antioxidants activities and decreased levels of lipid peroxides were seen in the brains of mice pretreated with *E.ganitrus* convincingly demonstrated that the extract significantly reduces oxidative stress. Our study findings are in accordance with previous studies done by Reddy VVM et al [29] and Bagewadi HG et al[30].It is also established that Flavonoids content in *E.ganitrus* leaves has marked antioxidant activity[31]. The anti-parkinsonian an effect of *E.ganitrus* may be attributed to the presence of alkaloids and flavonoids having marked antioxidant

and anti-inflammatory properties. In the present study ethanolic extract of *E.ganitrus* fruit has shown positive results in both behavioral parameters-1.Rota rod test (increase in retention time) and 2. Catalepsy bar test (decrease in latency period) in MPTP induced Parkinson's disease in mice. However, further studies should be undertaken to identify and investigate the mechanism of action of active anti parkinsonian compounds in *E.ganitrus.* The behavioral assessment of motor imbalance, catalepsy and antioxidant activity of *E.ganitrus* has to be further explored in other experimentally induced Parkinsonism models like Reserpine, 6-OHDA (6-Hydroxy dopamine). In conclusion, the results of the present study demonstrate that *E.ganitrus* has antioxidant activity and showed positive results in both behavioral parameters (Rota rod test and catalepsy bar test) in MPTP induced experimental model of Parkinson's disease. *E.ganitrus* found to be effective in increasing Rota rod performance and decreasing catatonic response. The neuromodulatory effect of *E.ganitrus* on behavioral, oxidative stress may be due its antioxidant properties. In this regard, future studies on this topic may enlighten to use *E.ganitrus* in clinical medicine for the treatment of Parkinson's disease.

REFERENCES

[1]. Ates DA, Erdogrul OT. Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol 2003;27:157-62.

[2].Singh RK, Acharya SB, Bhattacharya SK. Pharmacological activity of *Elaeocarpus Sphaericus*. Phytother Res 2000; 14:36-39.

[3].Singh RK, Bhattacharya SK, Acharya SB. Study of extract of *Elaeocarpus ganitrus* on in Vitro rat mast cells. Phyto Medicine 2000; 7:205-07.

[4]. Katavic PL, Venables DA, Rali T, Carroll AR. Indolizidine alkaloids with delta opioid Receptor binding affinity from the leaves of Elaeocarpus fuscoides. J Nat Prod 2007; 69:1295-99.

[5].Bhattacharya SK, Debnath PK, Pandey VB, Sanyal AK. Pharmacological investigations on *Elaeocarpus ganitrus* . Planta Medica 1975; 28:174-77.

[6].Agid Y, Ruberg M, Javoy-Agid F, Hirsch E, Raisman-Vozari R, Vyas S, et al. Are Dopaminergic neurons selectively vulnerable to Parkinson's disease? Adv Neurol 1993; 60:148-164.

[7].Prasad KN, Cole WC, Kumar B. Multiple antioxidants in the prevention and treatment of Parkinson's disease. J Am Coll Nutr 1999; 18:413-423.

[8]. Kakalij RM, Alla CP, Kshirsagar RP, Kumar BH, Mutha SS, Diwan PV. Ameliorative Effect of *Elaeocarpus ganitrus* on gentamicin-induced nephrotoxicity in rats. Indian J Pharmacol 2014; 46:298-302.

[9].Bagewadi HG, Afzal Khan AK. Evaluation of anti-parkinsonian activity of *Elaeocarpus ganitrus* on haloperidol induced Parkinson's disease in mice. Int J Basic Clin Pharmacol 2015; 4:102-6.

[10].Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm.Assoc 1955; 46:208-209.

[11].Hoffman DC, Donovan H. Catalepsy as a rodent model for detecting antipsychotic drugs with extrapyramidal side effect liability. Psychopharmacology 1995; 120:128-133.

[12].Ohkawa H, Ohishi N, Yaki K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351-358.

[13].Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-77.

[14]. Jenner, P. Preclinical evidence for neuroprotection with monoamine oxidase-B inhibitors in Parkinson's disease. Neurology 2004; 63:S13-S22.

[15].Yuan H, Zheng JC, Liu P, Zhang SF, Xu JY, Bai LM. Pathogenesis of Parkinson's disease:oxidative stress, environmental impact factors and inflammatory processes. Neurosci Bull 2007; 23:125-130.

[16]. Slivka A, Cohen G. Hydroxyl radical attack on dopamine. J Biol Chem 1985; 260:15466-15472.

[17].Sriram K, Pai KS, Boyd MR, Ravindranath V. Evidence for generation of oxidative stressin brain by MPTP: in vitro and in vivo studies in mice. Brain Res 1997; 749:44-52.

[18].Nair V, Arjuman A, Gopalakrishna HN, Nandini M.Effect of Withania somnifera root extract on haloperidol-induced catalepsy in albino mice. Phytother Res 2008; 22:243-246.
[19].Mohanasundari M, Srinivasan MS, Sethupathy S, Sabesan M. Enhanced neuroprotective effect by combination of bromocriptine and Hypericum perforatum extract against MPTP-induced neurotoxicity in mice. J Neurol Sci 2006; 249:140-144.
[20]. Polydoro M, Schroder N, Lima MN, Caldana F, Laranja DC, Bromberg E, et al. Haloperidol and clozapine induced oxidative stress in the rat brain. Pharmacology Biochem Behav 2004; 78:751-756.
[21].Aubin N, Curet O, Deffois A, Carter C. Aspirin and salicylate protect against MPTP- induced dopamine depletion in mice. J Neurochem 1998; 71:1635-1642.
[22].Teismann P et al.COX-2 and Neurodegeneration in Parkinson's disease. Ann N Y Acad Sci 2003;991:272-277.
[23].Singh RK, Pandey BL. Anti-inflammatory activity of Elaeocarpus sphaericus fruit extract in rats. J Med Arom Plant Sci1999;21:1030-32.
[24].Singh B, Chopra A, Ishar MP, Sharma A, Raj T. Pharmacognostic and antifungal investigations of *Elaeocarpus ganitrus* (Rudrakasha). Indian J Pharm Sci 2010;72:261-65.
[25]. Lal C. Tabulated phytochemical reports. Phytochemistry 1975;14:2727-28.
[26]. Johns SR, Lamberton JA, Suares H, Sioumis AA, Willing RI. The alkaloids of Elaeocarpus sphaericus. Australian journal of chemistry 1971; 24:1679-94.
[27]. Ray AB, Chand L, Pandey VB. Rudrakine, a new alkaloid from *Elaeocarpus ganitrus* . Phytochemistry 1979; 18:700-01.
[28]. Chand L, Dasgupta S, Chattopadhyay SK, Ray AB. Chemical investigation of some Elaeocarpus species. Planta Medica 1977; 32:197-99.
[29]. M.Vishnu Vardhan Reddy, Manmohan Singhal. Evaluation of Anti-Parkinsonian Activity of seed extract of Nelumbo Nucifera. Int J Pharm Bio Sci 2014;5:469-485.
[30].Harish G Bagewadi, Naveen Rathor. Effect of *Aloe vera* on animal models of Parkinson disease in mice. Int J Pharm Bio Sci 2014;5(3):549 – 559.
[31]. Sathish Kumar T, Shanmugam S, Palvannan T, Bharathi Kumar VM. Evaluation of Antioxidant Properties of *Elaeocarpus ganitrus* Roxb. Iranian Journal of Pharmaceutical Research 2008; 7:211-15.