EVALUATION OF ANTI PARKINSON’S ACTIVITY OF NIGELLA SATIVA (KALONJI) SEEDS IN CHLORPROMAZINE INDUCED EXPERIMENTAL ANIMAL MODEL

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INTRODUCTION

Parkinson’s disease (PD) is a slowly progressive neurodegenerative disease caused when a small group of brain cells that control body movements die. This disease was first described by James Parkinson in 1817. It is characterized clinically by bradykinesia, resting tremor, rigidity and postural instability. Pathological features of PD include loss of dopamine neurons in substantia nigra and the presence of Lewy bodies in surviving dopamine neurons [1]. The disease occurs in about 1% of the people over the age of 65 years. The peak onset of the disease is in the sixth decade of the life. The available treatments are levodopa, carbidopa, apomorphine, Amantadine, orphenadrine, benzhexol, benzotropine, selegiline, pergola and many more. These drugs effectively reverses the symptoms of Parkinson and improves the level of dopamine. The available drug treatments for PD possess various side effects like nausea and vomiting, depression, respiratory disturbances, hallucinations, mania, convulsions and anxiety, arrhythmia, mydriasis, orange discoloration of saliva and urine, dyskinesia on long term use, postural hypotension, peripheral vasospasm, ankle edema, nervousness, insomnia, constipation, dry mouth, sore throat, transient dizziness, diarrhea and abdominal pain, sleepiness, increased hunger. The major side effects of long term therapy with levodopa are wearing off phenomenon, on-off phenomenon and dyskinesia [2].

Nigella sativa (N. Sativa) seeds have an immense medicinal value [3] and are known to have numerous medicinal properties, mainly in the Unani-Tibbi/Greco-Arab and Ayurveda systems of medicine. It belongs to family Ranunculaceae. They contain about 21% protein, 35% carbohydrates and 35-36% plant fats and oils [4]. Specific chemical analyses of the volatile oil started during the years 1960-1963 [5], [6]. These studies were completed by most recent ones which revealed various pharmacologically active constituents that included Thymoquinone that may attain up to 27.8% of the volatile oil (w/w), Carvacrol 5.8-11.6% (w/w), 4-cymene in the range of 15.5-31.7% (w/w), alpha-pinene (9.3%), 4-terpinen 2-6.6%, longifolene 1-8% (w/w), t-anethole benzene 0.25-2.3% (w/w) [7], [8], [9], [10]. Traditionally, they have been used as diuretic, diaphoretic, stomachic, immunomodulator [11], [12] liver tonic and digestive [13]. Nigella sativa has been widely used in neurodegenerative diseases like Parkinson and Alzheimer from the past because of its antioxidant potential [14]. It contains thymoquinone (an antioxidant) having potential to protect brain from neurodegeneration as free radicals are most important cause of neuronal cell death [15]. Experimental studies have shown various central nervous actions like anxietyolic by elevated plus maze, the light/dark test and the social interaction tests in mice [16], anticonvulsant by using pentylentetrazole and maximum electroshock model [17], antioxidant agent [18] against Propoxur-induced toxicity [19], Anti Alzheimer by Morris water maze (MWM) test and Neuro Protectant using Cerebrovascular hypoperfusion [20] in animals.

MATERIAL AND METHOD

Plant material

Ethanolic extract of Nigella sativa seeds was procured from Amsar Private Limited 47, Laomibai Nagar, Fort, Industrial estate, Indore 452006, Madhya pradesh, India. The plant was identified and authenticated by Dr. Vikram Naharwar, Technical, Amsar private limited.

Experimental animals

Adult Wistar rats of either sex, weighing 150–200 gm were procured from Sanjay Biologics, Opp. Hindu Sabha Sr. Sec. School, Dhab Khatikan, Amritsar, Punjab-143001. Before and during the experiment, the animals were maintained in a well-ventilated room with a 12-hour light/dark cycle in standard poly propylene cages [43 × 27 × 15 (l × b × h) cms] under controlled temperature (26 ± 1°C) and humidity (30%-40%). They were fed with a standard pellet diet obtained from Gold Mohor, Lipton India Ltd, Hyderabad and water ad libitum throughout the experimental period. All animal experiments were carried out in accordance with the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) [21] and study was approved by the IACUC (Institutional animal ethical committee) with proposal no. RIP/IACU/2012-13/10.

Acute toxicity study

Wistar rats weighing 150-200 g (three male animals) were used in the study. Acute oral toxicity was performed as per the OECD-423 guidelines. The animals were fasted overnight, provided with water after which EENS was administered orally at a dose level of 5 mg/kg, p.o. intubation. If mortality was observed in two or three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality [22], [23].
Experimental design

Adult male Wistar rats (150–200gm) were divided into four groups each containing six animals. Group I received the vehicle 1% gum acacia solution and served as the control, group II III, IVa and IVb received chlorpromazine (3 mg/kg, i.p.). Group II receiving chlorpromazine only served as the negative control without any drug treatment [24], [25]. Group III received combination of L-dopa and Carbidopa (10 mg/kg, i.p.) and served as standard group [26] and Groups IVa and IVb received ethanolic extract of Nigella sativa at doses of 200, 400 mg/kg body weight, respectively for 21 days. Chlorpromazine was given 30 minutes prior to standard and test drug. Catalepsy was induced by the intraperitoneal administration of chlorpromazine at a dose of 3 mg/kg body weight in 1% gum acacia suspension. All the behavioural studies were performed at room temperature in a calm room without any external interference. After the 21 days, animals were sacrificed by cervical dislocation and the whole brain was immediately dissected out and washed in ice-cold saline to remove all traces of blood. The brains were weighed and a 10% tissue homogenate was prepared in 0.1 M Potassium Phosphate pH 8 for the activities of Thioharbituric acid reactive substances (TBARS), reduced glutathione (GSH), Nitrite and Total protein.

Behavioral studies

Measurement of catalepsy by block method [27] This scoring method was followed in three steps.

Step 1: 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.

Step 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.

Step III: The front paws of the rats 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 scores of steps I and II. Thus, the highest score for any animal was 3.5 (cut off score) and that reflects total catalepsy [28].

Neurochemical studies

Dissection and homogenization

Chronic chlorpromazine treated animals on day 22nd after behavioural quantification were sacrificed by cervical dislocation. 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 1 for each paw was added to the scores of steps I and II. Thus, the highest score for any animal was 3.5 (cut off score) and that reflects total catalepsy [28].

Estimation of lipid peroxidation products

Lipid peroxidation was estimated spectrophotometrically in brain tissue by quantifying TBARS [29]. In brief, for the estimation of TBARS the supernatant of the tissue homogenate was treated with Thiobarbituric acid - Tri chloro acetic acid, (TBA–TCA) reagent and mixed thoroughly. The mixture was kept in boiling water bath for 15 minutes. After cooling, the tubes were centrifuged for 10 minutes and the supernatant taken for measurement. The developed color was read at 532 nm using a UV spectrophotometer against a reagent blank.
The concentration of TBARS in the supernatant was determined from the standard curve using 1,1,3,3- Tetra Methoxy Propane (TMP) and expressed in nM/mg of protein [30].

Estimation of reduced glutathione (GSH)

1 ml of tissue homogenate was precipitated with 1 ml of 10% TCA. The precipitate was reprecipitated with 1 ml of 10% TCA.

Statistical analysis

All values are expressed as mean ±SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett’s multiple comparison tests, and other data was evaluated using Graph Pad PRISM software. A P-value <0.05 was considered significantly different.

RESULTS

Acute toxicity

The EENS did not produce any toxic symptom or mortality up to a dose level of 2000 mg/kg body weight orally in rats, and hence the drugs were considered safe for further pharmacological screening. According to OECD 423 guidelines further increment of doses could not be possible due to less solubility of the extract. So, the two dose ranges 200 mg/kg and 400 mg/kg were selected.

Effect of chronic administration of EENS on CPZ induced catalepsy

The cataleptic scores of the present study are given in Table 1 and Figure 1 assessed by block method. Chlorpromazine induced catalepsy significantly (P<0.001) at a dose of 3 mg/kg (intraperitoneal administration). Significant reversal in chlorpromazine-induced catalepsy was observed, with the administration of ethanolic extract of Nigella sativa and combination of L-dopa and carbidopa. The maximal decrease (P<0.001) in catalepsy was observed in the groups receiving ethanolic extract of Nigella sativa at a dose of 200 and 400 mg/kg.

Table 1: It shows Effect of Nigella sativa on catalepsy in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>Catalepsy score (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control Group)</td>
<td>Gum Acacia 1%</td>
<td>0</td>
</tr>
<tr>
<td>II (Negative Control)</td>
<td>CPZ (3mg/kg, i.p.)</td>
<td>1.727 ± 0.148 a***</td>
</tr>
<tr>
<td>III (Standard Group)</td>
<td>Levodopa+Carbidopa (10 mg/kg, i.p.) and CPZ (3mg/kg, i.p.)</td>
<td>0.387 ± 0.038 b***</td>
</tr>
<tr>
<td>IVa (Test 1)</td>
<td>EENS (200mg/kg,p.o) + CPZ (3mg/kg, i.p.)</td>
<td>0.780 ± 0.040 b***</td>
</tr>
<tr>
<td>IVb (Test 2)</td>
<td>EENS (400mg/kg,p.o) + CPZ (3mg/kg, i.p.)</td>
<td>0.587 ± 0.017 b***</td>
</tr>
</tbody>
</table>

a*= P<0.05, a**= P<0.01, a***= P<0.001 as compared to control group (Group I) [Groups II is compared with Group I]

b*= P<0.05, b**= P<0.01, b***= P<0.001 as compared to Chlorpromazine treated negative control group (Group II) [Groups III, IVa and IVb are compared with Group II]
Catalepsy

Fig. 1: It shows Effect of \textit{Nigella sativa} on catalepsy in rats

\[ a^* = P < 0.05, a^{**} = P < 0.01, a^{***} = P < 0.001 \text{ as compared to control group (Group I)} \]

\[ b^* = P < 0.05, b^{**} = P < 0.01, b^{***} = P < 0.001 \text{ as compared to Chlorpromazine treated negative control group (Group II)} \]

Effect of chronic EENS on the brain MDA or TBARS level in chronic CPZ treated rats

Chronic CPZ treatment to rats for 21 days induced lipid peroxidation as indicated by a significant \((P < 0.001)\) rise in brain MDA levels compared with the vehicle treated rats. Chronic administration of LD+CD \((10 \text{ mg/kg p.o.})\) and EENS \((200 \text{ and } 400 \text{ mg/kg})\) to CPZ treated animals significantly \((P < 0.05)\) and \((P < 0.001)\) respectively reversed the extent of lipid peroxidation compared with CPZ only treated rats (Table 2) (Figure 2).

Reduced Glutathione

Fig. 2: It shows Effect of \textit{Nigella sativa} on the TBARS level in rat brain

\[ a^* = P < 0.05, a^{**} = P < 0.01, a^{***} = P < 0.001 \text{ as compared to control group (Group I)} \]

\[ b^* = P < 0.05, b^{**} = P < 0.01, b^{***} = P < 0.001 \text{ as compared to Chlorpromazine treated negative control group (Group II)} \]

Effect of chronic EENS on the brain glutathione (GSH) levels in chronic CPZ treated rats

Statistical analysis of brain GSH levels showed a significant difference \((P < 0.001)\) between the vehicle treated and CPZ treated rats. However, chronic administration of EENS \((200 \text{ and } 400 \text{ mg/kg})\) showed a significant increase \((P < 0.5)\) and \((P < 0.001)\) respectively in the level of GSH compared with CPZ treated rats (Table 2) (Figure 3).

Nitrite

Fig. 3: It shows Effect of \textit{Nigella sativa} on the GSH level of rat brain

\[ a^* = P < 0.05, a^{**} = P < 0.01, a^{***} = P < 0.001 \text{ as compared to control group (Group I)} \]

\[ b^* = P < 0.05, b^{**} = P < 0.01, b^{***} = P < 0.001 \text{ as compared to Chlorpromazine treated negative control group (Group II)} \]

Effect of chronic EENS on the brain nitrite level in chronic CPZ treated rats

Chronic CPZ treatment to rats for 21 days induced lipid peroxidation as indicated by a significant \((P < 0.001)\) rise in brain Nitrite levels compared with the vehicle treated rats. Chronic administration of EENS \((200 \text{ and } 400 \text{ mg/kg})\) to CPZ treated animals significantly \((P < 0.01)\) and \((P < 0.001)\) respectively reversed the increase in nitrite level compared with CPZ only treated rats (Table 2) (Figure 4).

Fig. 4: It shows Effect of \textit{Nigella sativa} on the Nitrite level in rat brain

\[ a^* = P < 0.05, a^{**} = P < 0.01, a^{***} = P < 0.001 \text{ as compared to control group (Group I)} \]

\[ b^* = P < 0.05, b^{**} = P < 0.01, b^{***} = P < 0.001 \text{ as compared to Chlorpromazine treated negative control group (Group II)} \]

Effect of chronic EENS on the total protein levels in chronic CPZ treated rats

CPZ treated groups indicated a significant \((P < 0.001)\) decrease in total protein content (Table 2) (Figure 5) when compared with the vehicle treated group. Which was significantly increased by the EENS treated groups when compared with CPZ treated group \((P < 0.5)\) and \((P < 0.001)\) respectively.
Table 2: It shows Effect of Nigella sativa on TBARS, GSH, Nitrite, Total Protein in rats

<table>
<thead>
<tr>
<th>Gp</th>
<th>Drug treatment (mg/kg, route)</th>
<th>TBARS (nM/mg protein)</th>
<th>GSH (nM/mg protein)</th>
<th>Nitrite (nM/mg protein)</th>
<th>Total Protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Gum Acacia 1%</td>
<td>2.123 ± 0.132</td>
<td>10.087 ± 0.227</td>
<td>2.090 ± 0.470</td>
<td>10.876 ± 0.305</td>
</tr>
<tr>
<td>II</td>
<td>CPZ (3mg/kg, i.p.)</td>
<td>4.311 ± 0.096 a***</td>
<td>4.177 ± 0.312</td>
<td>8.371 ± 0.308 a***</td>
<td>4.345 ± 0.119 a***</td>
</tr>
<tr>
<td>III</td>
<td>LD + CD (10 mg/kg, i.p.) and CPZ (3mg/kg, i.p.)</td>
<td>3.148 ± 0.059 b***</td>
<td>8.762 ± 0.172</td>
<td>3.983 ± 0.299 b***</td>
<td>8.197 ± 0.375 b***</td>
</tr>
<tr>
<td>IVa</td>
<td>EENS (200mg/kg, p.o) + CPZ (3mg/kg, i.p.)</td>
<td>3.88 ± 0.059 b**</td>
<td>5.784 ± 0.248</td>
<td>5.130 ± 0.315 b**</td>
<td>5.662 ± 0.336 b*</td>
</tr>
<tr>
<td>IVb</td>
<td>EENS (400 mg/kg, p.o) + CPZ (3mg/kg, i.p.)</td>
<td>3.406 ± 0.059 b***</td>
<td>7.644 ± 0.187</td>
<td>4.473 ± 0.232 b***</td>
<td>7.482 ± 0.284 b***</td>
</tr>
</tbody>
</table>

a* = P<0.05, a** = P<0.01, a*** = P<0.001 as compared to control group (Group I) [Groups II is compared with Group I]

**DISCUSSION**

Parkinson’s disease is a neurodegenerative disorder characterized by the selective loss of dopamine (DA) neurons of the substantia nigra pars compacta. The events which trigger and/or mediate the loss of nigral DA neurons, however, remain unclear. Parkinsonism refers to a clinical syndrome characterized by a variable combination of rest tremor, bradykinesia or akinesia, cogwheel rigidity, and postural instability. Current treatment of Parkinson’s disease (PD) is based on dopamine replacement therapy, but this leads to long term complications, including dyskinesia. Plants pose an important and a safer alternative to the treatment of neurodegenerative disorders including Parkinsonism.

In the present study we evaluated the effect of Nigella sativa, a plant traditionally used for Parkinson’s disease, in rodents using chemical inducer of Parkinson’s, chlorpromazine.

Chlorpromazine induced catalepsy is a widely accepted animal model of Parkinson’s disease. Some authors have demonstrated that chlorpromazine provides a pharmacological model of Parkinsonism [34] by interfering with the storage of catecholamines in intracerebral granules, resulting in monoamine depletion (norepinephrine, 5-hydroxytryptamine and dopamine) in nerve terminals [35]. Antipsychotic effects and extrapyramidal symptoms are also produced due to dopamine depletion. In the present study, Chlorpromazine (3 mg/kg, i.p.) induced significant catalepsy in rats as evidenced by a significant increase in the time spent on the block as compared to the vehicle treated control rats. Treatment with Nigella sativa, a neuroprotectant, dose dependently reduced the catalepsy in chlorpromazine-treated rats. The protective effect of Nigella sativa on the doses of 200 and 400 mg/kg against chlorpromazine induced catalepsy suggested that this plant has influence on the amnergic receptor mediated neurotransmission.

Animal data have demonstrated elevated oxidative stress markers with 21-days administration of haloperidol or chlorpromazine, but not atypicals. With chronic dosing in rats for 21-days, chlorpromazine is associated with the greatest level of oxidative stress and increased lipid peroxidation. There was a significant increase in catalepsy, decrease in movements and decrease in body weight following chlorpromazine administration to rats. The current data thus suggested damage to motor control system (DA-ergic neurons) and development of Parkinson’s disease like behavioral symptoms in chlorpromazine treated rats. The oxidative stress was measured through determination of levels of TBARS (or MDA), reduced glutathione, nitrite and Total protein.

The extent of lipid peroxidation was estimated by measuring the levels of thiobarbituric acid, a product of lipid peroxidation. Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function, impaired structural integrity [36], decreased fluidity and inactivation of number of membrane bound enzymes. There is substantial evidence of oxidative damage in the brains of PD patients. Increased levels of the lipid peroxidation product, thiobarbituric acid have been found in the substantia nigra of PD patients [37]. Similar results were observed in the brain homogenates of chlorpromazine-treated negative control animals.

A defect in one or more of the naturally occurring antioxidant defenses particularly GSH is an important factor in etiology of PD [38]. A reduction in GSH levels may impair H2O2 clearance and promote hydroxyl radical formation leading to the generation of pro-oxidant milieu.

Nitrate and nitrate determinations in biological material are increasingly being used as markers of NO production. We detected nitrite in the rat brain homogenates by the Griess method [39]. Nitric oxide production was quantified by measuring nitrite, a stable oxidation end product of NO [40].

Chlorpromazine significantly induce catalepsy as compared to control group. Chlorpromazine per se group also showed a significant increase in the levels of thiobarbituric acid (which is an indication of extent of lipid peroxidation) and in the levels of nitrite. Whereas, a decrease in Reduced glutathione and Total Protein was observed in the brain as compared to the vehicle treated control animals. All these indicate an increase in the oxidative stress in the brain of animals treated with chlorpromazine. Nigella sativa significantly reduce cataleptic scores showing its anti cataleptic activity. Pretreatment with Nigella sativa also resulted in a decrease in TBARS and nitrite level, increase in glutathione and Total Protein level. This suggested that Nigella sativa acts by increasing activity of antioxidant or decreasing oxidation and thus may contribute to increased availability of GSH to act against increased oxidative stress and decreased level of TBARS and Nitrite showing decrease in free radical induced oxidation.

Since, oxidative stress produced in brain due to chlorpromazine toxicity seems to be important in producing motor defects, therefore use of antioxidants could prove beneficial. The present study which thus explored the potential of Nigella sativa (earlier proved to be an antioxidant by [41]) in neurodegenerative disorders showed a promising effect in animals with Parkinson’s disease or symptoms. Thymoquinone, is already claimed to be an antioxidant. Although fractions rich in thymoquinone were found to be most potent in terms of antioxidant capacity, however, previous research indicates that the protective effects of Nigella sativa may not only be due to thymoquinone, but perhaps also due to other antioxidants [42].

Further studies with more models of Parkinson’s and on different extracts and fractions of the plant to further explore and identify more chemical constituents responsible for neuro protective effect need to be carried out.

**CONCLUSIONS**

Nigella sativa was found to possess a therapeutic effect against Parkinson’s disease in chlorpromazine induced animal PD models. Further studies with different extracts and their fractions are encouraged to identify the chemical constituents responsible for Anti-Parkinson’s activity. Also clinical studies to prove this effect is...
also needed for its applicability in humans for treatment of Parkinson’s disease.

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