Effect of *Eclipta alba* and *Ocimum sanctum* on haloperidol induced parkinsonism

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**ABSTRACT**

The aim of the study is protective effect of compound Eclipta alba and Ocimum sanctum on Parkinsonism induced mice by haloperidol injection. Parkinsonism is neurodegenerative disease due to the deficiency of dopamine in brain. The pathological hallmark of Parkinson’s disease in the cell loss within substantia nigra pars compacta (SNpc) region and the disease is characterised by bradykinesia, rigidity, postural instability, orofacial dyskinesia, muscular stiffness and tremor. Mice were injected 1mg/kg haloperidol and then treated with test and standard substance for 15 days. The impairment in catatonia in mice were tested using catatonic activity. Biochemical analysis of brain homogenate was performed so ass to assess brain Thiobarbituric acid reactive substance (TBARS) level and reduced glutathione (GSH) and TNF-α level were measured to assess total oxidative stress. EA 300mg/kg and OS 400mg/kg show slightly change in catatonic activity in mice while EA 600mg/kg and 800mg/kg significantly change in catatonic activity. Furthermore, Eclipta alba and Ocimum sanctum prevent the haloperidol induced changes in the level of brain TBARS, GSH and TNF-α. From the results we conclude that Eclipta alba and Ocimum sanctum has protective action against impairment in catatonic activity and pathological damage due to oxidative stress induced by intraperitoneally injection of haloperidol in mice.

**Keywords:** *Eclipta alba*, *Ocimum Sanctum*, Parkinsonism, Anti-oxidant.

**INTRODUCTION**

Neurodegenerative diseases were believed to be incurable and debilitating conditions, which primarily affected the neurons in the human brain resulting in the loss of nerve structure and function and ultimately leading to the death of nerve cells. The major characteristic features of neurodegenerative diseases include ataxias (impairment in movement) and dementia (decline in memory). The three main types of neurodegenerative diseases that affect the life quality and life span of the elderly include Alzheimer’s disease (AD), Parkinson’s disease (PD), and Huntington’s disease (HD). Haloperidol (HP) belongs to the group of typical incisive antipsychotics. It is highly potent drug used particularly in the management of acute states, such as psychosis, manic phases, hyperactivity, aggressiveness, and acute delirium; in some cases, it is administered on a long-term basis. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen and are produced in all aerobic cells. Oxidative stress occurs when the generation of ROS in a system exceeds that system’s ability to neutralize and to eliminate them. All organisms have developed adaptive responses to oxidative stress that involve defensive enzyme and molecular chaperones—the expression of both being orchestrated by stress-responsive transcription factors—as well as antioxidant molecules. Antioxidants mainly act by:

1. Inhibiting the NADPH oxidase and reduce NADPH oxidase-mediated generation of reactive oxygen species.
2. Balance NO production from different NO synthase isoforms.

3. Reducing neuroinflammation via attenuation of the release of cytokines and down regulation of the pro-inflammatory transcription factors.

4. Modulating signaling pathways such as mitogen-activated protein kinase cascade and cAMP response element binding protein are responsible for the neuroprotective actions of different natural polyphenols

**MATERIALS AND METHODS**

Swiss albino mice of either sex weighing 25±2g were employed in present study. The animals were housed in the departmental animal house and were exposed to 12hr light and dark cycle. The animals were acclimatized to the laboratory conditions before experiments. The experimental protocol was duly approved by the institutional animal ethical committee.

**Preparation of Aqueous extract of E. alba**

The entire, dried plant was then coarsely powdered and the purity and quality of the crude drug was established as per the procedures laid down in Indian Pharmacopoeia 1996. The powdered plant material was then subjected to extraction with water by decoction for 18 h in Soxhlet’s extractor, to obtain the aqueous extracts. The yield of aqueous extract (Aq. Ext.) was found to be 31.44% (w/w). Preliminary phytochemical investigations conducted as per the procedures described by Kokate (1994) and Trease and Evans (1997) of the extracts revealed the presence of saponins, tannins, carbohydrates and phenolic compounds.

**Preparation of alcoholic extract of Ocimum sanctum**

The leaves of OS were collected, authenticated, air dried and powdered. The extract was prepared from this powder (1 kg) by percolation with 70% ethyl alcohol at room temperature and was then concentrated under reduced pressure (below a temperature of 50 °C). It was finally vacuum dried in a lyophiliser (Herysun Instruments Co.). The yield of the extract was 1 g/100 g powder. This extract was dissolved in Tween 80 for administration in animals.

**Administration of extract of E. alba and Ocimum Sanctum**

The test substances were administered to animals by oral gavage as a single dose using oral gavage tube. The dosage volume administered to individual mice was adjusted according to its body weight recorded on the day of dosing. The animals were administered with 300mg/kg body weight.

**Drug and Chemical**

Bromocriptine (Proctinal®, 2.5mg/Tablet, Glaxo Smithkline Pharmaceuticals Ltd, Mumbai, India) suspended in tween 80, were given orally and used as reference standard drugs in this study. Haloperidol (Serenace®, 5mg/ml, RPG Life Sciences Ltd, Mumbai, India).

**Brain Tissue Sampling and Preparation**

After completion of behaviour tests, the animals were kept fasting for 12 hours. Blood samples were collected using the orbital sinus technique of Sandford. The mice were killed by decapitation and the whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried and then weighed. The brain was homogenized with ice-cold 0.1M phosphate buffer (pH 7.4, 10% (w/v). The homogenate was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant (10%) was separated for biochemical analysis.

**Methodology for catatonic mice**

Various stages of catatonia were induced in mice with haloperidol. The standard and test substance were administered 30min prior to haloperidol injection and scoring was made at 15, 30, 45, 60 and 90 after the injection. The development and severity of the four stages of induced catatonia were observed and score as follow:

**Stage I:** Rat moves normally when placed on the table, score=0.

**Stage II:** Rat moves only when touched or pushed, score=0.5.

**Stage III:** Rat placed on the table with front paws set alternatively on 3cm high block fails to correct the posture in 10 sec, score=0.5 for each paw with a total of 1 for this stage.

**Stage IV:** Rat fails to remove when the front paws are placed alternatively on 9cm block, score= 1 for each paw a total score of 2 for this stage.

Thus, for a single mouse, the maximum possible score would be 3.5 revealing total catatonia and less score would mean an apparently lesser degree of catatonia.

The day after completion of catatonic response mice were sacrificed and biochemical parameters were estimated like Brain Dopamine, Brain Thioaributiric acid reactive substance (TBARs), Brain reduced Glutathione (GSH), Locomotor activity, alteration in these parameter shows the activity of the test and standard drugs.

**Experimental Design**

**Group I (Catatonia control):** Catatonia control group were treated with 1mg/kg Haloperidol intraperitoneally.

**Group II (Standard-1 treated group):** Standard 1 group were infected with 1mg/kg Haloperidol to induce catatonia in mice and treated with Bromocriptine 30mg/kg/day and code as SD-I

**Group III (Test dose-1 treated group):** Test-1 group were infected with 1mg/kg Haloperidol to induce catatonia in mice and treated with extract of E. alba 300mg/kg/day and code as test-1

**Group IV (Test dose-2 treated group):** Test-2 group were infected with 1mg/kg Haloperidol to induce catatonia in mice and treated with extract of E. alba 600mg/kg/day and code as test-2
Group V (Test dose-3 treated group): Test-3 group were infected with 1mg/kg Haloperidol to induce catatonia in mice and treated with extract of OS 400mg/kg/day and code as test-3

Group VI (Test dose-4 treated group): Test-4 group were infected with 1mg/kg Haloperidol to induce catatonia in mice and treated with extract of OS 800mg/kg/day and code as test-4

RESULTS

Effect on Catatonic behavioral

It was observed that haloperidol 1mg/kg administered i.p alone treated group, significantly increased the catalepsy 15th day as compared to vehicle treated group. In Bromocriptine treated group significantly decrease in catalepsy score (p<0.001) was seen 15th day, as compared to haloperidol treated group. EA 300mg/kg and OS 400mg/kg 30 min. pretreated groups did cause slightly change in catalepsy score on 15th day. When compared with standard treated group. But on 15th day, whereas EA 600mg/kg and OS 800mg/kg 30 min. pretreated group was marked decrease in catalepsy score (p<0.001) when compared with standard treated group in dose dependent manner.

![Figure 1: Effect on Catatonic behavioral](image1)

Effect on Brain TBARS Activity

Haloperidol treated group significantly increase the TBARS activity on 15th day as compare to normal group and reflecting enhanced oxidative stress. Treatment with EA 300mg/kg and OS 400mg/kg per se group had slightly decreased in TBARS activity as compare to catatonic control group. But when it was compared with standard groups their results are slightly increase, whereas EA 600mg/kg and OS 800mg/kg treated group significantly abolished the haloperidol induced rise in brain oxidative stress level in dose dependent manner.

![Figure 2: Effect on Brain TBARS Activity](image2)
Effect on Brain GSH Activity

Haloperidol treated group significantly reduce the glutathione activity on 15th day as compare to normal group and reflecting enhanced oxidative stress. Treatment with EA 300mg/kg and OS 400mg/kg per se group had slightly increased in glutathione activity as compare to catatonic control group. But when it was compared with standard groups their results are slightly decrease in activity. Whereas EA 600mg/kg and OS 800mg/kg treated group significantly abolished the haloperidol induced rise in brain oxidative stress level and significantly increase the level of glutathione in dose dependent manner.

![Figure 3: Effect on Brain GSH Activity](image)

Effect on Brain TNF-α Activity

Haloperidol treated group significantly increase in TNF-α activity on 15th day as compare to normal group and reflecting enhanced oxidative stress. Treatment with EA 300mg/kg and OS 400mg/kg per se group had slightly decreased in TNF-α activity as compare to catatonic control group. But when it was compared with standard groups their results are significantly higher in TNF-α activity. Whereas EA 600mg/kg and OS 800mg/kg treated group significantly abolished the haloperidol induced rise in brain oxidative stress level and significantly decrease the level of TNF-α in dose dependent manner.

![Figure 4: Effect on Brain TNF-α Activity](image)
DISCUSSION

In our study, mice were selected to induce catalepsy with haloperidol due to blockage of striatal D2 receptor\textsuperscript{12}.

Haloperidol remains effective in inducing catalepsy and striatal Fos/Jun expression in the D1 mutants, and these behavioral and neural effects can be blocked by D2 dopamine receptor antagonists\textsuperscript{13}. Catalepsy occurs when more than 80% of D2 receptors are occupied by the drug\textsuperscript{14}. Catalepsy is also driven by the excitatory adenosine and glutamatergic inputs acting on adenosine A2A and N-methyl-D-aspartate (NMDA) receptors in the striatum. This is because NMDA receptor antagonists\textsuperscript{15}. The previous data shows that Maximum cataleptic score is observed after 60 and 90 min of administration. The free radical’s production can be inactivated by free radical scavenging system. In PD, substantia nigra is conductive to the formation of cytotoxic free radicals. These free radicals cause lipid peroxidation and cell death, which shows that it leads to a severe oxidative state in SN. Increased oxidative stress leads to over consumption of SOD, GPX and show movement disorders\textsuperscript{16}, so in the present study it is necessary to assess the cataleptic score. In our study cataleptic score was increase with intraperitoneally injection of haloperidol for 15 days and test and standard drugs was given 30 min prior to the haloperidol injection. From the results test drugs shows that cataleptic score was decrease or come close to standard drug.

Several studies show that depletion of the antioxidant peptide glutathione (GSH) in PD cells, which may be caused by a decrease in its synthesis and recycling\textsuperscript{17}. The hallmark of idiopathic Parkinson's disease (PD) is loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to the major clinical and pharmacological abnormalities that characterize the disease. The most robust and significant alteration in the antioxidant defense is a decrease in GSH concentration. Initially, a complete absence of GSH in the presence of high GSSG concentrations was reported. However, in the brain most of the GSH is localized in glia. The levels of glutathione transferase, a protective enzyme against aldehydes and especially HNE were decreased in the brain and ventricular CSF of autopsied AD and normal control subjects\textsuperscript{18}. In our study GSH activity is decrease by haloperidol in different groups due to oxidative stress or generation of free radicals. From the results GSH activity in test drugs show same or come close to standard drug i.e standard 1 in dose dependent manner.

Haloperidol (HP) is converted to potentially toxic (HHP+) metabolites which may play a role in the extrapyrimidal side effects which are observed in the patients who are treated with haloperidol. Another possible mechanism could be the effect of neuroleptics on the mitochondrial respiration. The metabolites of haloperidol inhibit complex-I of the electron transport chain. The capability of the anti-psychotic drugs to clinically induce the extra-pyramidal syndrome seems to correlate well with their inhibitory effect on the complex-I inhibition. Whatever could have been be the mechanism of the unbalanced production of the reactive oxygen species (ROS) and the oxidative stress by haloperidol\textsuperscript{19}. Increased TBARS activity has been shown to be an important marker for in vivo lipid peroxidation in the learning and memory deficient mouse brains. In addition, it has been reported that haloperidol significantly increases TBARS activity in the hippocampus and frontal cortex\textsuperscript{11, 20}. From the results we know that standard-1 decrease the TBARS level in brain, but test substance reduces the level of TBARS in dose dependent manner.

On treating with haloperidol resulted in significantly increases in the level of TNF-α in the cortex and striatum region of the mice brain. Many cellular studies indicated that haloperidol has been associated with an increased expression of inflammatory markers such as TNF-α and NF-kB against oxidative damage induced by haloperidol that may lead to neurotoxicity\textsuperscript{21, 22}. However, with chronic haloperidol, there is a downregulation of phospho-Thr34-DARPP-32 because of an increase in TNFα signaling. In the CNS, the effects of TNFα are complex, with evidence for both injury-promoting and neuroprotective effects. Moreover, TNFα levels are elevated in patients and animal model of movement disorder, such as HD, PD, and other basal ganglia disorders, such as OCD, schizophrenia, and addiction. Overall, our data establish that TNFα is a key element in maintaining the normal function of this circuit and that the robustness of striatal compensatory systems may set the symptom susceptibility in these maladies\textsuperscript{23}. When measured in whole brain, levels of TNF-α were decreased but when studied in selective striatal regions as in our study, levels were significantly increased\textsuperscript{24}. So, it is necessary to find TNF-α activity in present study. In our study haloperidol injection 1mg/kg decrease in TNF-α after 30min. of test-(1,2,3,4) and standard-1. From the results test drugs show increase or come close to standard-1 in dose dependent manner.
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