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Original Research Article

Neuro-Protective Influence of Lutein on Haloperidol-Induced Parkinson Disease in Wistar Rats

Austin A. Ajah[1]*, Precious C. Eton[1], Chike CPR[1]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Nigeria

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***Corresponding author:** Austin A. Ajah
Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Nigeria

Abstract

Parkinson's Disease (PD) is a debilitating neurodegenerative disorder characterized by motor deficits and dopaminergic neuron degeneration. Haloperidol, a widely used antipsychotic, has been reported to induce parkinsonism-like symptoms in animal models. This study investigates the potential neuroprotective effects of lutein, a carotenoid with antioxidant properties, against haloperidol-induced Parkinson's disease in Wistar rats. A total of Thirty (30) healthy Wistar rats weighing between 100 -150g were used for this study. The rats were acclimatized and divided into six groups (n=5 per group); Group 1 (Control), Group 2 (Haloperidol group), Group 3 (Haloperidol + 20mg/kg of lutein), Group 4 (Haloperidol + 40mg/kg of lutein), Group 5 (Haloperidol + 60mg/kg of lutein) and Group 6 (Haloperidol + donepezil group). Motor deficits were assessed using behavioral tests like Barnes maze test, hand grip test, rotarod test and Y maze test. While biochemical analyses were performed to evaluate oxidative stress markers. The results demonstrated that rats treated with haloperidol alone had a significantly higher latency compared to the control group. This suggests that haloperidol negatively impacted spatial learning and memory, as indicated by the increased time taken to find the target. Lutein, especially at 40mg/kg and 60mg/kg, as well as the standard drug donepezil were seen to have neuroprotective effects against the spatial learning and memory deficits induced by haloperidol in Wistar rats. This study showed that Haloperidol induced both the motor symptoms such as muscle rigidity and also the non-motor symptoms of Parkinson disease such as anxiety, oxidative stress, and impaired memory on the rats and lutein possesses a dose-dependent increase in learning ability and cognitive functions and decrease in oxidative stress and fatigue.

Keywords: Neuro-Protection, Lutein, Haloperidol-Induced, Parkinson Disease.

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INTRODUCTION

Parkinson disease is a progressive neurodegenerative disorder that is chronic and significantly impacts the central nervous system (CNS). It is characterized by a range of motor and non-motor symptoms mainly caused by the destruction of dopaminergic cells in the substantia nigra, which is located in the mid-brain part of the brain (Alberico *et al.*, 2015). This disease has far reached impacts on the lives of the affected patients, their families, and the health-care system, and thus it deserves serious consideration as a public health burden.

In addition to motor presentations, Parkinson disease has a deep effect on the central nervous system in the form of non-motor symptoms. Mood disorders, depression, anxiety, etc. are common, and they occur due to neurochemical imbalances as well as the psychological trauma of the disease progression. REM sleep behavior disorder and insomnia are also prevalent and lower the quality of life of the people who have them. The disease may further be complicated by cognitive impairment varying between mild cognitive impairment to dementia that interfere with memory, attention and executive functions (Aarsland *et al.*, 2012).

It is interesting to note that haloperidol, a common antipsychotic medicine, has been known to cause parkinsonism in certain patients, as symptoms of Parkinson disease. Its neurotoxicity is related to the disruption of the dopaminergic transmission and stimulation of oxidative stress and neuroinflammation (Valvassori *et al.*, 2021; Irokosu *et al.*, 2025). Haloperidol causes Parkinson-like symptoms (motor impairment and destruction of dopaminergic neurons) in animal models and is useful in investigating the mechanisms of neurodegeneration (Irokosu *et al.*, 2025).

Therefore, it is important to identify possible interventions to overcome the damage caused by haloperidol not just to learn more about the mechanisms of drug-induced parkinsonism but also to develop new neuroprotective modalities.

Lutein is a carotenoid antioxidant that is rich in green leafy vegetables, and it has been identified to have antioxidant properties. There is empirical evidence that it has the potential to reduce oxidative injury to the neurons and mitigate the inflammatory responses (Vishwanathan *et al.,* 2014). Moreover, it has the ability to cross the blood brain barrier and amass in the central nervous system, which suggests that it may have a neuroprotective effect (Arab Firozjae *et al.,* 2024). Despite this, it has not been investigated in detail in terms of its effects in the haloperidol-induced Parkinson disease. Since the neurotoxicity of haloperidol has been well established and neuroprotective effects of lutein have been documented, the role of lutein in modifying the action of haloperidol induced Parkinson disease in animal models is a major step towards determining its therapeutic value.

An enzyme known as catalase which catalyzes the process of degrading hydrogen peroxide is a critical line of defence against oxidative stress (Poewe *et al.,* 2017). Changes in catalase activity give an idea about the oxidative stress state of the brain. Nitric oxide is a signalling molecule that may perform a dual role in neurodegeneration as a neuroprotective neurotransmitter and a source of oxidative stress. Along with the excessive production of NO, which is a common phenomenon associated with inflammation and causes neurodegeneration, neurotoxicity in Parkinson disease can be enhanced (Wang *et al.,* 2023).

The most important question that should be answered is whether lutein, a carotenoid antioxidant that is antioxidant and anti-inflammatory, can exert neuroprotective effects against the neurotoxicity of haloperidol. Despite its established antioxidant effects, its particular effect on Parkinson disease induced by haloperidol has not been studied in details.

MATERIALS AND METHODS

Animal Model

Thirty (30) healthy Wistar rats weighing between 100 -150g were used for this study. The rats were obtained from the animal house of the Faculty of Basic medical sciences, Abuja campus, University of Port Harcourt and acclimatized to the laboratory environment for a period of 14 days before the commencement of the experiments. They were maintained under standard laboratory conditions with a 12-hour light/dark cycle, controlled temperature (22 ± 2°C), and ad libitum access to water and standard laboratory rodent chow.

Ethical Approval

Ethical approval was obtained from the faculty of basic medical science, Abuja campus, University of Port Harcourt. Rat handling and treatment conform to the guideline of the National Research Council (2011) for care and use of laboratory animals.

Chemicals and Reagents

The chemicals and reagents used for this study were purchased from GGI Intl' Nigeria Ltd. located at GGI Place, Plot 8 GGI Crescent, (Opp. Mikab Filling Station), Port Harcourt, Rivers State, Nigeria. The chemicals and reagents include:

Lutein: High-purity lutein extract was obtained in three different concentrations to create low(20mg), medium(40mg), and high (60mg) lutein dose groups. It was administered oral;
Haloperidol: Haloperidol, a commonly used antipsychotic drug, was used to induce Parkinson's disease-like symptoms in the rats. It was administered inter peritoneally;
Donepezil: Donepezil, an acetylcholinesterase inhibitor, served as the standard drug for comparison. It was also administered via I.P;
Sterile saline: Sterile saline solution was used for dilution of lutein and as a vehicle for drug administration;
Anesthesia: Isoflurane was used to anesthetize the rats during surgical procedures;
Biochemical assay kits: To measure oxidative stress markers, such as malondialdehyde (MDA) and superoxide dismutase (SOD), Catalase and Nitric oxide;
Apparatus for behavioral tests: Including the rotarod apparatus, Tail suspension test, barnes maze test, hand grip test, Y maze test, and elevated plus maze test;
Surgical instruments: For stereotaxic surgeries and tissue sampling;
Standard laboratory supplies: Including syringes, needles, vials, and other consumables;
Protective Equipments: Lab coats, Hand gloves, Face masks.

Experimental Design

The study consisted of 30 Wistar rats, randomly divided into six groups (n=5 per group) as follows:

1. **Control Group**: Rats received food and water only. Rats in this group served as the baseline comparison and did not receive any Haloperidol or lutein treatment. They were used to assess the natural state of cognition and balance in rats.
2. **Haloperidol-induced Group**: Rats were administered haloperidol to induce Parkinson's-like symptoms. However, they will not receive any lutein treatments. **1ml** of haloperidol was administered interperitoneally for 21 days. This group will help establish the negative effects of Haloperidol on motor functions.

3. **20mg/kg of Lutein Group**: Rats in this group received haloperidol to induce Parkinson's disease symptoms and they received 20mg/kg of lutein. Typically, a low dose of lutein for Wistar rats could range from around 1 to 20mg/kg. This group received lutein at a dose of 20mg/kg daily for 21 days. The purpose is to evaluate the potential beneficial effects of lutein at a low dosage on Parkinson symptoms.
4. **40mg/kg of Lutein Group:** Rats in this group were administered with haloperidol and received 40mg/kg of lutein for 21 days. The group aim is to investigate whether a slightly higher lutein dosage would have more pronounced impact on the neuro-protective function.
5. **60mg/kg of Lutein Group:** Rats in this group were administered with haloperidol and received 60mg/kg of lutein for 21 days. The objectives is to assess the effects of a higher dosage of lutein on neuro-protective function
6. **Donepezil Group:** Rats in this group were administered haloperidol to induce Parkinson's-like symptoms and received donepezil as a standard drug. Donepezil, an acetylcholinesterase inhibitor, served as the standard drug for comparison. It was administered via I.P

Drug Administration

The Administration was done over a 21-day period.

- **Haloperidol Administration:** Haloperidol a typical antipsychotic medication, was used to induce parkinsonian symptoms in the rats, mimicking Parkinson's disease. The selected dosage (1mg/kg/day, I.P.) was based on previous studies that reported motor and neurochemical changes similar to those seen in Parkinson's disease.
- **Lutein Treatment:** Lutein was orally administered. The stock solution was calculated based on the weight of the rats. Lutein was dissolved in 2ml of DMSO and stock solution was stored in EDTA bottle and was kept in the refrigerator (at 4°C) to protect it from light and maintain stability. This stock solution was stored in light-protected vials to prevent degradation. Prior to administration, the appropriate volume of the stock solution was diluted in 8ml of distilled water to achieve the desired treatment doses (20 mg/kg,40 mg/kg,60 mg/kg respectively) for the various lutein treatment groups. The treatment was initiated concurrently with haloperidol administration to assess its potential neuroprotective effects.
- **Donepezil Administration:** A standard drug with known neuroprotective properties, was used as a positive control. It was also administered intraperitoneally to the rats to assess its efficacy in mitigating the effects of haloperidol-induced Parkinson's disease.

Behavioural Assessment

Throughout the experimental period, rats were monitored daily for any adverse effects, including changes in behavior, body weight, anxiety and overall health. Motor dysfunction was assessed using various behavioral tests, including the rotarod test, open field test, and Hand grip test, to evaluate Parkinson's-like symptoms. While Y maze test Elevated plus maze test and Barnes test were used to evaluate Anxiety levels.

- **Rotarod Test:** The rotarod test was conducted to assess the motor coordination and balance of rats (Shiotsuki *et al.*, 2010). The latency to fall from the rotating rod was recorded.
- **Open Field Test:** The open field test was employed to evaluate the locomotor and exploratory activities of the rats. The rats were placed in an open field arena, and parameters such as total distance moved, time spent in the center, and rearing activity were recorded.
- **Hand Grip Test:** This test was used to evaluate the rats' motor coordination and muscle strength. It provided valuable insights into how lutein treatment influenced the rats' physical abilities in the context of Parkinson's disease.
- **Y Maze Test:** The Y maze test was employed to assess the rats' spatial working memory and cognitive function. It allowed researchers to determine whether lutein had a positive effect on the cognitive aspects of Parkinson's disease in the Wistar rats.
- **Elevated Plus Maze Test:** This test was utilized to examine the rats' anxiety-like behavior and their response to lutein treatment. It helped in understanding the potential anxiolytic effects of lutein in the context of Parkinson's disease.
- **Barnes maze Test:** The Barnes test was used to evaluate the rats' spatial learning and memory abilities. The test was named after Dr. Carol Barnes who developed it in 1979. By employing this test, researchers could investigate whether lutein had a significant impact on the rats' cognitive performance and spatial memory in the Parkinson's disease model.

Biochemical and Molecular Assessments

At the end of the experiment, rats were euthanized, and their brain tissues were collected for biochemical analysis. Biochemical assays were conducted to measure oxidative stress markers, inflammatory cytokines, and antioxidant enzyme activities. Blood samples were also collected for biochemical assessments.

Catalase and nitric oxide levels were chosen as biomarkers for their relevance to oxidative stress and neuroinflammation, key factors in Parkinson's disease progression. Biochemical assays and

immunohistochemical techniques were employed to quantify catalase activity and nitric oxide levels in the brain tissues of each group.

Statistical Analysis

All data obtained from the various assessments were subjected to appropriate statistical analyses. Statistical analysis was conducted using GraphPad Analysis software and the results were graphically represented using bar charts, providing a clear visualization of the effects of lutein on various parameters.

RESULTS AND DISCUSSIONS

Tables represent Mean ± SEM, n=5; * Indicates statistical significance of haloperidol and treatments versus the Negative controls. * Significant at P < 0.05, ** significant at 0.01, *** significant at 0.001 and **** significant at 0.0001.

Table 1: Neurobehavioural Outcomes Following Lutein Administration in Haloperidol-Induced Parkinson Disease Model (Wistar Rats)

	Negative Control	Haloperidol Only	Haloperidol + Lutein (20mg/kg)	Haloperidol + Lutein (40mg/kg)	Haloperidol + Lutein (60mg/kg)	Haloperidol + Donepezil (Standard Drug)
Barnes Maze Primary Latency	40.88 ± 1.72	72.73 ± 6.55 ***	64.00 ± 4.76 **	54.07 ± 3.69	49.00 ± 2.88	52.99 ± 2.39
Handgrip Stability Time	8.84 ± 2.26	46.34 ± 5.06**	21.88 ± 4.06	17.94 ± 7.54	29.33 ± 9.55	18.37 ± 3.96
Rotarod Stability Time	7.85 ± 3.37	29.26 ± 4.60*	16.62 ± 3.56	13.88 ± 6.13	19.08 ± 6.12	11.51 ± 1.94
Y Maze Inflexion Ratio	0.87 ± 0.15	1.22 ± 0.09	1.44 ± 0.23	1.26 ± 0.13	1.34 ± 0.11	1.48 ± 0.08
Y Maze % Spontaneous Alternation	31.69 ± 1.94	12.20 ± 0.66 ****	24.80 ± 1.36	27.80 ± 2.27	33.40 ± 1.94	31.80 ± 1.02

Table 2: Effects of Lutein on Catalase and Nitric Oxide in Haloperidol-Induced Parkinsonism (Wistar Rats)

	Negative Control	Haloperidol Only	Haloperidol + Lutein (20mg/kg)	Haloperidol + Lutein (40mg/kg)	Haloperidol + Lutein (60mg/kg)	Haloperidol + Donepezil (Standard Drug)
Catalase	0.88 ± 0.04	1.71± 0.05****	1.36 ± 0.15***	1.05 ± 0.01	1.03 ± 0.02	0.67 ± 0.12
Nitric Oxide	2.71 ± 0.11	4.61 ± 0.44**	4.06 ± 0.22	3.36 ± 0.22	3.10 ± 0.39	2.74 ± 0.20

DISCUSSION

Neurobehavioural Tests

- **Barnes Maze Test:** Haloperidol significantly increased the latency in finding the hole to escape, which meant that spatial learning and memory were impaired. The administration of lutein in dose dependent manner decreased latency; 40mg/kg and 60mg/kg of lutein came close to the latency recorded in the control group. Donepezil had similar effects to those produced by the 40mg/kg lutein group. These results are consistent with those of (Prathyusha *et al.,* 2025), who confirmed the antioxidant properties of lutein in the prevention of cognitive impairment.
- **Hand Grip Test:** Haloperidol prolonged grip stability by about four times compared to the control, thus, proving the effect of rigidity associated with parkinsonism. Lutein reduced grip time to normal in a dose-dependent manner and 40mg/kg of lutein was the most effective. Donepezil had similar results. In research conducted by Saeed *et al.,* (2017), Haloperidol reliably produces catalepsy and motor deficits in rodents, consistent with the marked prolongation of grip latency. While donepezil can improve some sensori-motor outcomes, its effects on motor function vary by model; therefore, the apparent similarity between 60mg/kg of lutein and donepezil should be interpreted with caution. (Cutuli *et al.,* 2013)
- **Rotarod Test:** Haloperidol treatment significantly extended the stability time because of muscle rigidity. 20mg/kg of lutein partially blocked this decline and 40mg/kg/60mg/kg doses completely restored the impairment to baseline performance. Donepezil had a corresponding reconstitution. These findings are similar to those provided by Hasumi & Maeda (2023) specifically zebrafish model, which proves that antioxidants and neurotransmitter-modulating compounds may reverse haloperidol-induced motor dysfunction.

- **Maze Inflexion Ratio:** Haloperidol increased inflexion ratio, which reflects the poor spatial working memory. Lutein suppressed this measure in all doses, but not to the baseline levels. The effect of donepezil on the index was not significant. This is in line with Xu *et al.,* (2012) research, where haloperidol is reported to impair spatial working memory. Findings can probably due to dosing regimens and the duration of treatment.
- **Maze Spontaneous Alternation:** Haloperidol produced the least percentage of alteration, hence validating cognitive impairment. On the other hand, 60mg/kg of lutein reinstated the performance to the control level, but 20mg/kg of lutein and donepezil only partially reinstated the performance.

Catalase Activity

Haloperidol elevated catalase activity compared to all other groups, indicating oxidative stress. On the other hand, Lutein inhibited catalase activity in a dose-dependent way.

Nitric Oxide Concentration

The administration of haloperidol was linked to a significant elevation of nitric oxide (NO) as compared to control. On the other hand, lutein decreased NO dose-dependently and 60mg/kg of lutein group produced the greatest effect as compared to the 40mg/kg of lutein group. The same decrease in NO was observed with donepezil.

Both tables indicate that lutein especially at 60mg/kg has a strong tendency of alleviating the cognitive impairment, motor deficits and oxidative stress caused by haloperidol. Its effects were equivalent or better than donepezil in a number of measures, which suggests that lutein may have a role as a neuroprotective adjunct in drug-induced parkinsonism and cognitive impairment.

CONCLUSION

The present study together with existing reports demonstrates the neuroprotective effects of lutein on haloperidol-induced Parkinson's disease in Wistar rats. The study focused on evaluating catalase and nitric oxide as biomarkers to understand the potential benefits of lutein in mitigating neurotoxicity associated with haloperidol-induced Parkinson's disease.

The study has shown that catalase activity was increased in haloperidol induced Parkinson's disease and this will help to protect cells from damage which are induced by reactive oxygen species and lutein decreases nitric oxide which plays important physiologic roles in lower concentrations. This study shows that Haloperidol induced both the motor symptoms such as muscle rigidity and also the non-motor symptoms of Parkinson disease which are fatigue, oxidative stress, and impaired memory on the rats. Lutein an antioxidant carotenoid possesses a dose dependent increase in learning ability and cognitive functions and decrease in oxidative stress and fatigue.

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