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Neuroprotective effect of moringa oleifera seed extract against haloperidol-induced catalepsy in wistar rats: A possible treatment against Parkinson's disease?

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ABSTRACT
Parkinson's disease is a leading neurological and neurodegenerative disorder troubling 1–3 % of the elderly population. Reactive oxygen species production is the main aspect for the neurodegeneration of substantia nigra. The recent study purposes to evaluate the effect of seed extract of moringa oleifera on haloperidol induced catalepsy in a rat model. For this purpose, ethanolic seed extracts of moringa oleifera was prepared, i.e. (EthMO). Male wistar albino rats were divided into six groups. Disease group received 1 mg/kg haloperidol for 21 consecutive days, and control mice received the normal saline. Treated rats received 100 mg/kg, 150 mg/kg and 250 mg/kg of EthMO separately, orally and daily for 28 days. Levodopa group received 20 mg/kg, intraperitoneally, as a positive group. The motor function was assed using open field and hole board test. The anti-oxidant potentials of moringa seed extract was estimated by lipid peroxidation (LPO), reduced glutathione activities in rat brain homogenates. The rat brain region of substantia niagra was taken out for gene expression. Moringa oleifera seed extract-treated rat exhibited a greater number of entries in open field test along with greater number the rat introduced their head in hole board test as compared to the haloperidol induced catalepsy group. Improved antioxidant level of the PD rat brains of MO extract-administered groups were observed compared to the control. Gene expression results showed reduction in the expression level of TNF- α, IL-1β moringa extract treated rats. On the contrary the gene expression of α-Synuclein was also diminished in the extract group as compared to the haloperidol treated group. It can be concluded that moringa seed extracts protected the animals from locomotor deficits induced by haloperidol, possibly through reduction in the neuroinflammation, and look to have potential claims in neurodegenerative diseases.

Keywords:
Parkinson's disease, haloperidol induced catalepsy, Moringa oleifera, TNF-α

1. Introduction

Parkinson's disease (PD) is a neurological and neurodegenerative disease characterized by the loss of dopaminergic neurons in Substantia Nigra pars compacta. and affects 1–3% of the world population. The manifestations of the disease include progressive loss of motor co-ordination and includes tremor, bradykinesia, postural instability and rigidity [Raza et al., 2019]. The Parkinson's disease is idiopathic but it is believed that oxidative stress is amongst the foremost causes of PD features with dopaminergic neuronal loss, neuroinflammation and dysfunctional mitochondria [Blesa et al., 2015]. The production of (ROS)

and clearance through antioxidant enzyme system operates in usual homeostasis. Nevertheless, increased ROS generation and disturbed antioxidant capacities have distressing effects on DA neurons. Oxidative insult cause distress and gradual degeneration of dopamine neurons of substantia niagra results in the insufficient release of dopamine [Trist et al., 2019]. Dopamine is the major neurotransmitter interceding motor activities from mid-brain basal ganglia, and an obvious decrease in its release is observed in PD patients compared to age-matched healthy individuals [Cheng et al., 2010]. A foremost cause of sporadic PD is mitochondrial dysfunction, predominantly losses in processes involved in energy production, such as the electron transport chain [Li et al., 2021].

While Parkinson's disease is not a lethal disease, as it greatly affects the quality of life of affected persons and causes an enormous socio-economic burden [Gumber et al., 2019]. Recent treatment and management approaches for this disease aim to slow down the disease progression and improve the quality of life [Raza et al., 2019]. Of note, operative treatment plan to prevent PD are presently lacking. Thus, it creates a need to introduce and develop such therapeutic agent from natural resources which have no or limited side effects.

Amongst many of the strategies, plant-derived natural products have gained the significant attention of scientists and pharmacologists for the handling of several diseases, including neurodegenerative disorders [Limanaqi et al., 2020]. Bioactive compounds from plant extracts have so far established countless precautionary and therapeutic potential. Polyphenols have shown weighty antioxidant potential through free radical scavenging and chelating properties [Renaud et al., 2029]. Several polyphenolic derivatives from plant extracts have demonstrated anti-parkinsonian properties by inhibiting the production of inflammatory factors, including nitric oxide (NO), prostaglandin E2 (PGE2) and tumor necrosis factor-α (TNF-α), as well as reducing dopaminergic neuronal loss [Shahpiri et al., 2016; Uddin et al., 2020].

Moringa oleifera is a perennial tree with huge medicinal benefits. It is found in northern India and Pakistan of the sub-continent South Asia. Moringa oleifera is cultivated in tropical and sub-tropical parts of the world as well and it is commonly known as *Sohanjana*. The seed extracts of MO show antioxidant properties in a cell-free medium [Jahan et al., 2018]. Recent studies report the presence of bioactive peptides (such as –Napin-1A-P24565) from the crude extract of MO seeds [Chandrashekar et al., 2020]. The supplementation with moringa oleifera seeds and the presence of bioactive compounds with antioxidant properties could safeguard the susbtantia niagra neurons of rotenone-induced PD mice and may reduce the extent of motor dysfunction. Therefore, the present study uses PCR approaches to evaluate the beneficial effects of MO seed extracts on a haloperidol-induced rat model of PD. This study discloses the protective potential of MO seed extracts on haloperidol-mediated motor functional damages in rat.

2. Materials and Methods

2.1. Plant Material

Moringa oleifera seeds were purchased from the local market of Karachi, Pakistan. The plant was identified by the taxonomist of GC University Faisalabad based on morphological characteristics, and an (herbarium number #1904).

2.2. Chemicals

All the chemicals used were analytical grade: Haloperidol (Cat. # 52-86-8, USA), ethanol (Cat. # 32221, RDH-Germany), DMSO (67-68-5, Carl Roth-Germany), cDNA (Cat. # K1621 kit Thermo Scientific).

2.3. Preparation of M. oleifera Seed Extracts

The seed kernels of M. oleifera were grounded to obtain seed powder without husk, ethanolic extracts of M. oleifera seeds were prepared according to the methodology previously defined [Jahan et al., 2018; Nafiu et al., 2019]. Briefly, 15 g of moringa oleifera seed dry powder obtained and sieved, then the powder was suspended in 350 mL of absolute ethanol and adjusted in an orbital shaker at 180 rpm. Filtration was performed using Whatman No. 1 filter paper. The volume of the filtrate was reduced at 42 °C, and lyophilized extracts were stored at 4 °C until used. Final yield of 13.97% were obtained from ethanolic extracts, respectively. The percentage yield of the extract was calculated using the following formula.

$$\text{Percentage Yield} = \frac{\textit{Final extract mass}}{\textit{Initial mass of seed powder}} \times 100$$

2.4. Experimental Protocol

Male wistar albino rats weighing 200–250 g was obtained from the animal facility of Dow University of Health Sciences. Animals were kept in standard cages at 26 ± 2 °C, 12 h light and dark cycles, with unrestricted supplies of diet and water. All the procedures were performed following Institutional Review Board approval from Salim Habib University.

Rats were divided into 6 groups (n = 10 rats per group). The control group were given normal saline. The Disease group were given 1 mg/kg intraperitoneal injection of haloperidol to induce PD symptoms [Ahmed et al., 2012]. Positive control group have received levodopa 20 mg/kg intraperitoneally. While the treatment groups were given moringa seed extracts orally in the dose of 100 mg/kg, 150 mg/kg and 250 mg/kg orally for 28 days respectively.

2.5. Behavioral Test

The rats were subjected to behavioral tests i.e. open field test and hole board test to observe PD motor symptoms. All the tests were recorded and analyzed under controlled conditions.

Open field test examines the locomotion function of rat and is widely used to observe the motor dysfunction following inception of the PD symptoms [Prasad et al., 2020]. Momentarily, rat was tagged with a black dot on head scruff dorsally and recorded in a 50 cm2 arena with a white paper sheet floor (marked in sixteen equal-sized squares). rat was place in the center

of the apparatus, and the rat were permissible to explore the apparatus for 5 min. After five minutes, the rat was returned to its cage, and 70% ethyl alcohol was used to clean the open field apparatus. The number of entries in squares, marked on the white floor, was manually counted from video recordings of 5 min duration.
The hole board test was done with an HBT apparatus. The apparatus comprised of a gray vinyl chloride box (40 cm × 40 cm × 35 cm) that had four holes in the floor. Each rat was placed inside the box, and the number of times the rat introduced its head in these holes was manually counted. The test lasted 5 min [Frausto et al., 2021].

2.6. Biochemical Estimation

The antioxidant markers were studied from haloperidol induced rat PD brains vs. PD brains from moringa oleifera seed extract-treated rat. After the dissection freshly procured rat brains were homogenized. The homogenate was used partially for lipid peroxidation, and the residual volume was suspended in phosphate buffer (0.1 M, pH = 7.4) and centrifuged (12,000× g at 4 °C for 25 min) to obtained supernatant.
Lipid peroxidation measurement was achieved by mixing 10% tissue homogenate with equal volumes of 0.67% trichloroacetic acid and 10% 2-thiobarbituric acid, boiled for 45 min in the water bath and centrifuged (6000× g for 10 min) as reported previously [Wright et al., 1981]. The absorbance at 532 nm was recorded from the separated supernatant. The LPO level was analyzed with the help of reactive compounds contained in the TBA.
Using the extinction coefficient of 1.56 × 105/M/cm at 37 °C, it was demonstrated as nmol/mg of tissue. The reduced glutathione (GSH) was quantified as described previously [Faheem et al., 2020]. A total of 10% PMS (200 µL) was incubated with an equal volume of 4% sulfosalicylic acid at 4 °C for 1 h and centrifuged (1200 rpm for 15 min) to collect the supernatant. The supernatant, 10 mM DTNB (5,5'-dithiobis-2-nitrobenzoic acid) and sodium phosphate buffer (0.1 M, pH 7.4) were mixed, absorbance at 412 nm was recorded.

2.7. RNA Isolation and RT-PCR

Rat brain samples substantia niagra region were taken in an eppendorf tubes and tissue homogenate was used. RNA was extracted from cells using trizol reagent (Ambion™, Life Technologies, USA) in accordance with the protocol provided by the manufacturer. The concentration and purity of RNA was assessed using Nanodrop 2000UV–VIS spectrophotometer (Thermo Scientific, USA). The isolated RNA was treated with DNase to remove any possible contamination of genomic DNA. cDNAs was prepared from 1 µg of RNA using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, USA) according to the protocol described by the manufacturer. The cDNAs were amplified using DreamTaq Green PCR Master mix (Thermo Fischer Scientific, USA) according to the manufacturer's protocol with GAPDH used as housekeeping gene. The primer sequences used in this study were TNF-α (forward, 5'-GGCGTGTTCATCCGTTCTCT-3') (reverse, 5'-AATTCTGAGCCCGGAGTTGG-3'), IL-1β (forward, 5'-TGGGATGATGACGACCTGC-3') (reverse 5'-ATTCTTCCCCTTGAGGCCC-3'), α- Synuclein (forward, 5'-TGGAGTGACAACAGTGGCTG-3') (reverse, 5'-CAGGATTCCCTCTTGTGGGT-3'), Gapdh (forward, 5'-ACCACAGTCCATGCCATCA-3') (reverse, 5'-TCCACCACCCTGTTGCTCT-3'), PCR products were resolved on agarose gel electrophoresis and visualized by ethidium bromide dye. The resolved bands were quantified using ImageJ software and were normalized to housekeeping gene.

2.8. Statistical Analysis

All the data were entered and assessed by statistical software, IBM SPSS Statistics data editor version 21.0. One–way ANOVA has been used to evaluate the values which were homogenous. The statistical differences ($p < 0.05$) were measured using one-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test to compare all means pairwise. The data were represented as mean ± SEM.

3. RESULTS

3.1. Open filed Test

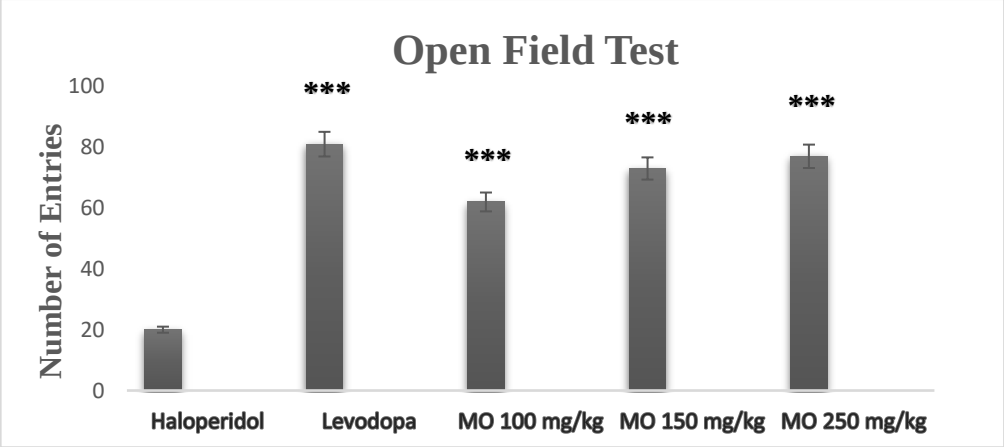


Figure:1 seed extract of moringa oleifera preserve the exploratory activities of haloperidol induced catalepsy. Open field trajectories of rats showed the exploratory activity revealed a reduction in locomotor activity of mice in haloperidol treated group; however, a higher extent of locomotor activity was found in MO extract treated and levodopa treated groups. Data

are represented as mean ± SEM; n = 10. ** p < 0.01, *** p < 0.001 One-way ANOVA followed by Bonferroni post hoc analysis.

3.2. Hole Board Test

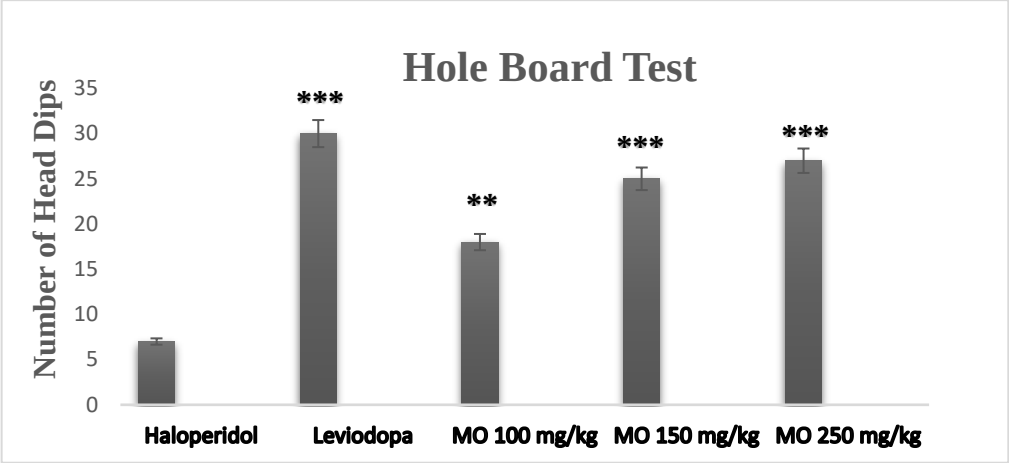


Figure:2 seed extract of moringa oleifera preserve the motor activities against haloperidol induced catalepsy. In the hole board test, the number of times the rat introduced their head in holes was measured and it significantly decreased in the haloperidol treated group compared to moringa extract and levodopa. Data are represented as mean ± SEM; n = 10. ** p < 0.01, *** p < 0.001 One-way ANOVA followed by Bonferroni post hoc analysis.

3.3. Biochemical Estimation

Table 3.1 showed that the haloperidol group represented the decreased levels of the glutathione as compared to the levodopa (standard) treatment group. On the other hand, the moringa Oleifera group showed the increased in the levels of glutathione. However, haloperidol group represented the lipid oxidation with an increased in the total lipids, as the treatment group with test extract showed to have decreased the lipid oxidation levels.

Groups	Glutathione mm/mg protein	Lipid Oxidation nmole/mg
Haloperidol 1 mg/kg	3.68+0.71	24.8+8.85*
Levodopa 20 mg/kg	10.31+0.28	7.63+0.36
Moringa Oleifera 100 mg/kg	12.85+2.89*	8.78+0.99
Moringa Oleifera 150 mg/kg	16.78+2.44**	6.02+1.13*
Moringa Oleifera 250 mg/kg	22.0+1.16***	3.49+1.13*

3.4. Gene Expression

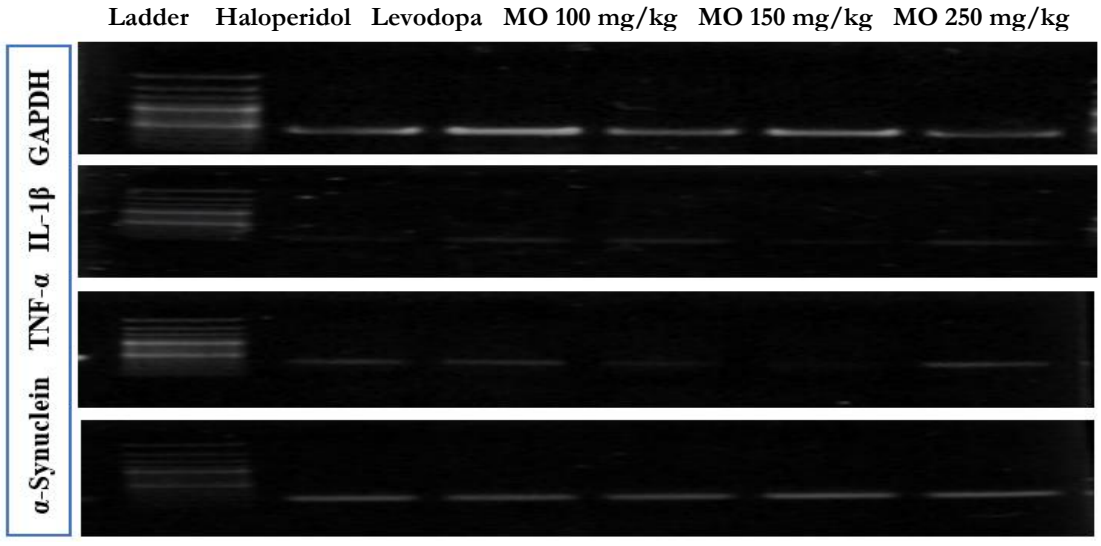
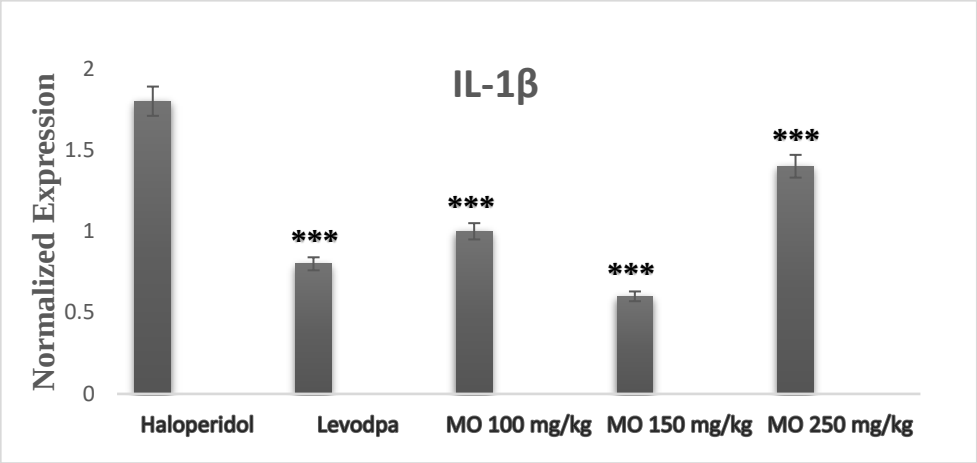
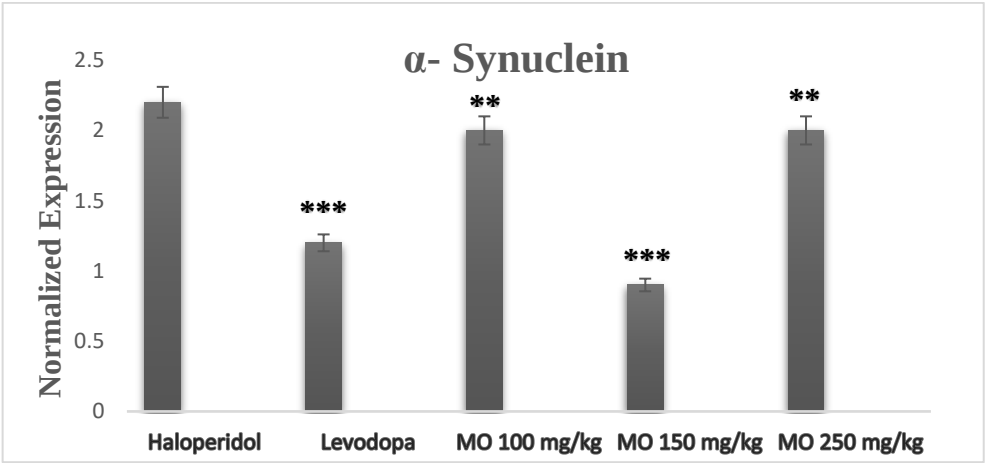


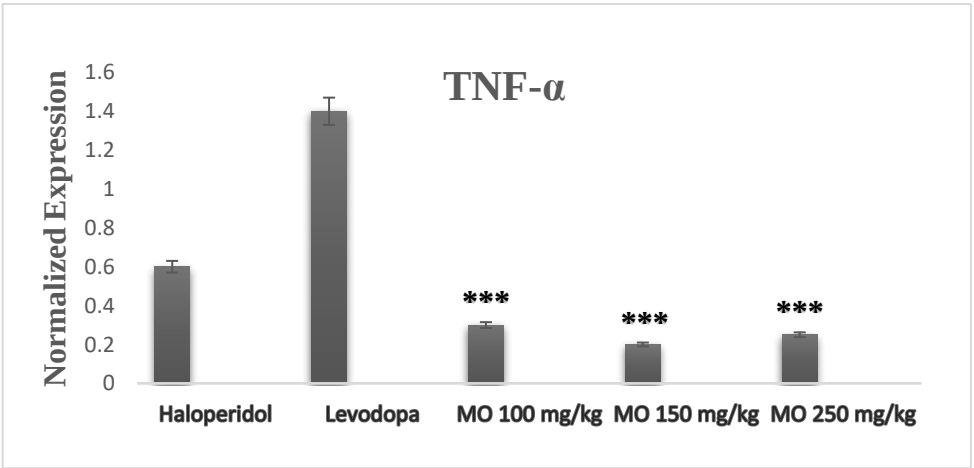
Figure: 3. Expression levels of IL-1, TNF-α and α- Synuclein mRNA in the brains of haloperidol, Levodopa, MO 100 mg/kg, MO 250 mg/kg and MO 250 mg/kg. Graphs showed that the gene expression of IL-1β, TNF-α and α-Synuclein was increased in the haloperidol treated rats, however the expression was diminished in the moringa oleifera extracts groups. This revealed the neuroprotective effect of the moringa extract in rats against PD symptoms.



Expression of IL-1β following treatment with Haloperidol, Levodopa, moringa oleifera 100 mg/kg, moringa oleifera 150 mg/kg, moringa oleifera 250 mg/kg. Data was analysed using one-way ANOVA and Dunnett's comparison test for significance. Each bar represents Mean + S.E.M. The statistical significance is shown as *P<0.05, **P<0.01, ***P<0.001.

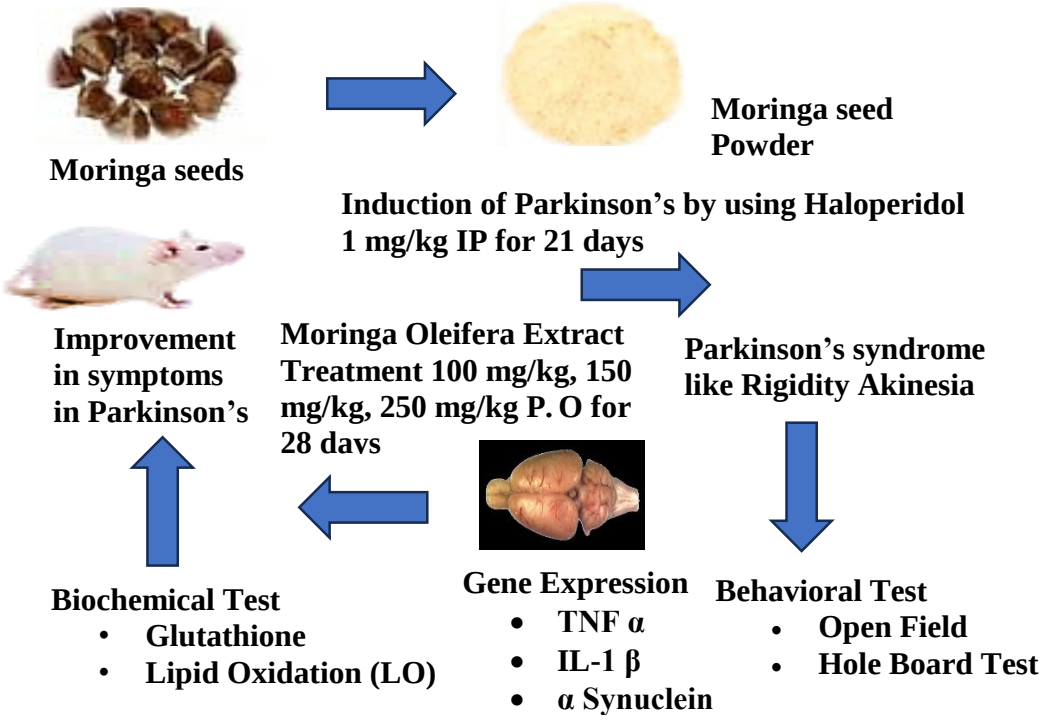


Expression of α-Synuclein following treatment with Haloperidol, Levodopa, moringa oleifera 100 mg/kg, moringa oleifera 150 mg/kg, moringa oleifera 250 mg/kg. Data was analysed using one-way ANOVA and Dunnett's comparison test for significance. Each bar represents Mean + S.E.M. The statistical significance is shown as *P<0.05, **P<0.01, ***P<0.001.



Expression of TNF- α following treatment with Haloperidol, Levodopa, moringa oleifera 100 mg/kg, moringa oleifera 150 mg/kg, moringa oleifera 250 mg/kg. Data was analysed using one-way ANOVA and Dunnett's comparison test for significance. Each bar represents Mean + S.E.M. The statistical significance is shown as *P<0.05, **P<0.01, ***P<0.001.

4. Discussion



Scheme 1. Schematic representation of Moringa oleifera seed extracts and their Anti- Parkinson's potential.

In present study, we intended to discover the effects of ethanolic extracts from Moringa Oleifera seeds extract on a haloperidol induced catalepsy in rat model. Muscle co-ordination, condensed exploratory behaviour and compromised dexterity and poise are the haloperidol mediated motor dysfunctions that were stated earlier [Raza et al., 2019]. The open field, test is commonly used to judge motor dysfunctions in Parkinson's pre-clinical models [Prasad et al., 2020; Mohammadzadeh et al., 2018]. This test assesses exploratory activities, motor coordination, balance, locomotion and immobility. In the current study, haloperidol induced PD rats which were followed by moringa oleifera extract treatment and levodopa administration confirmed improved exploratory activities compared to only haloperidol treated group in open field test. These above finding proposes that the useful role of extracts in sustaining normal locomotor functions and performance of control rats are comparable to that of the earlier report [Bonito-Oliva et al., 2014].

Oxidative stress is known to cause changes in iron, ferritin and metallic ion concentrations in dopaminergic neurons of substantia niagra [Bresgen et al., 2015]. As, oxidative stress encouraged nuclear translocation of nuclear factor-KB increases the risk (up to 70-fold) of dopaminergic cell death in PD [Faucheux et al., 2003]. Haloperidol induced mitochondrial complex I dysfunction is the main resources of ROS described to activate toxicity of integral dopaminergic neurons [Testa et al., 2005]. Thus, the investigation of the antioxidant potential of rat brains exposed to haloperidol and extract treatments is possibly the most applicable method to measure the defensive potential of tested drugs moderating the oxidative status of cells. We explored the antioxidant markers, reduced glutathione from rat brain homogenates. In the current study, the lipid peroxidation levels of haloperidol induced rat brains was raised, showing decreased antioxidant potential. nevertheless, a significant decrease of LPO in ethanolic moringa oleifera seed extractt treated group and levodopa treated rat portrayed the improved antioxidant capacities to clear free radicals. The exhausted levels of reduced glutathione (GSH) are testified from sustantia niagra of PD post-mortem brains [Pearce et al. 1997]. A recent study reported that GSH administration improves the motor functions of PD rats [Wang et al., 2021], signifying a possible role as a scavenging agent to counterbalance oxidative stress. We observed a huge increase in GSH levels in ethanolic extract and levodopa treated group. It has been reported that suboptimal (30–50%) levels of GSH in PD are due to suboptimal production [Fitzmaurice et al., 2003]. Of note, an augmented level of GSH is detected in the current study, suggesting that moringa oleifera ethanolic extract appears to protect GSH production in haloperidol treated rats.

The better motor functions and increased antioxidant levels in extract treated rat groups proposed the probable neuroprotection of the mid-brain neurons. haloperidol treatments led to the degeneration of sustantia niagra neurons in PD animal models [Cannon et al., 2009]. In our study, the degree of neurodegeneration in haloperidol treated rats treated with/without moringa extracts was explored.

After treatment with our extract the expression of TNF α and IL-1β were decreased which showed that compound decreasing the levels of these inflammatory markers and reduces the inflammation. Which were increased in the haloperidol treated rats. As neuroinflammation is one of the leading causes of neurodegeneration of dopaminergic neurons. However, the gene expression of α synuclein also declined in the treatment group as compared to disease group.

5. Conclusions

After the administration of moringa oleifera seed extract to haloperidol treated rats-maintained motor functions as evaluated through a neurobehavioral test. The rat brain homogenates subsequent in vivo moringa seed extract treatment completion portrayed substantial anti-oxidant potential through increased levels of reduced glutathione activities compared to PD rats. The gene expression results revealed neuroinflammation has been reduced through decreased in the expression of IL-β and TNF-α. The levels of α-Synuclein were also decreased in the extract treated rats. In the present study, the prevention of motor dysfunctions in MO seed extract-treated rats under haloperidol exposure appears to be possibly through the antioxidant potential of the extract ingredients. The results of the current study recommend that future studies should be conducted to discover the single active ingredient from the extract with conceivable neuroprotective capacities. Together, the current data point to the therapeutic potential of Moringa Oleifera seed extracts with possible clinical applications.

Conflicts of Interest

The authors declare no conflicts of interest.

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