Modulation of haloperidol-induced catalepsy in wistar rats by foxtail millet (*Setaria italica*)

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Abstract

The current study looked at the behavioral and antioxidant activity of Foxtail Millet (FM) against haloperidol-induced catalepsy in Parkinson's Disease (PD) patients. It has been demonstrated that the antipsychotic drug haloperidol, which has a high capacity to block D2-type receptors, can cause motor impairments similar to those seen in people with PD. Catalepsy can develop when animals are placed in abnormal or unusual postures for an extended period of time. Foxtail millet significantly reduced lipid peroxidation (p 0.001) increased the antioxidant enzymes SOD (p 0.05) and GSH (p 0.05), and significantly improved motor deficits such as catalepsy, motor coordination, and locomotor activity in our study. These results show that foxtail millet can protect against the motor deficits (catalepsy) associated with PD and epilepsy.

Introduction

One of the most common neurodegenerative movement disorders is Parkinson's Disease (PD). PD is expected to double in global prevalence by 2040, surpassing Alzheimer's disease as the neurological ailment with the fastest rate of growth. PD manifests itself in several clinical forms (akinetic, rigid, and tremulous) and causes a variety of symptoms, including the motor triad (tremor, rigidity, and bradykinesia). The progressive death of dopaminergic neurons in the Substantia Nigra pars compacta (SNc) causes movement dysfunction in PD, and new research suggests that oxidative stress is a major trigger for the intricate degenerative cascade that underpins dopaminergic neurodegeneration in all types of PD. When the production of Reactive Oxygen Species (ROS) exceeds the removal by endogenous antioxidant enzymes and molecular chaperones, oxidative stress occurs.[1] Haloperidol-treated rats exhibit symptoms similar to PD. A typical neuroleptic drug, haloperidol, works by blocking postsynaptic dopamine D2 receptors[2] in the mesolimbic system. This inhibiting effect causes an increase in dopamine turnover. Haloperidol also has minor anticholinergic and -adrenergic receptor blocking properties. PD is characterized primarily by the loss of melanin-containing dopaminergic neurons in the substantia nigra's zona compacta.[3]

Haloperidol causes marked rigidity, which is most likely due to a potent blockade of central dopamine receptors[3] and decreased dopamine neurotransmission.[4] Neurotoxins such as MPTP, 6-OHDA, and haloperidol are commonly used in preclinical studies to create experimental models of PD[5] that can model specific aspects of the disease such as motor abnormalities and catalepsy. Only when dopaminergic neuronal death exceeds a critical threshold of 70-80% of striatal nerve terminals do clinical symptoms appear.[3] Catalepsy is a symptom of certain nervous disorders such as PD and epilepsy. Muscular rigidity, loss of muscle control, slowing of bodily functions, and wavy flexibility are among the symptoms. Catalepsy occurs when animals are placed in abnormal or unusual postures that they maintain for an extended period of time. A normal animal will return to its normal position and explore its environment within seconds, whereas a cataleptic animal will maintain this externally imposed posture for an extended period of time.[6]

Foxtail Millet (FM) was the first whole grain cultivated by humans (*Setaria italica L.*).

FM is high in phenolic acids, minerals, fiber, protein, and other phytonutrients.[7] It has consistently gained popularity, owing to its hypoglycemic, hypolipidemic, and antioxidant properties.[8] Because of their low side effects and long history of human use, natural products derived from the diet are known to exhibit a variety of biological effects.[9] FM extracts exhibited changes in GABA[10] levels as well as anti-proliferative[11] and antioxidant[12] activities due to the presence of polyphenols. So, we hypothesized in this study that FM could cause significant changes in the motor deficits caused by haloperidol.

Materials and Methods

Foxtail millet powder preparation and administration

The FM was purchased at a local market, ground to a fine powder, sieved, and stored in an airtight container.

The FM powder was finely ground and mixed with powdered rat feed in two different ratios: low dose (25%w/w) and high dose (50%w/w). The FM powder was mixed with rat feed (75% w/w) and (50% w/w) by adding tap water 33, formed into pellets, and given to two groups separately as normal rat feed along with water for 28 days after disease induction. The method was used in accordance with the protocol developed by Ren *et al.*[8]

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Chemicals

Sigma-Aldrich, India, supplied the haloperidol at a concentration of 1mg/kg. All other necessary chemicals were obtained from commercial suppliers in the area and were of analytical grade.

Animals

Mahaveer Enterprises in Hyderabad, India, provided male young Wistar albino rats weighing 200-250 g. Under standard laboratory conditions, the animals were housed in polyacrylic cages. The rats were kept on a 12-hour light/12-hour darkness cycle, and they were fed water and rat chow pellets as needed.

Behavioral experiments were conducted from 10 a.m. to 4 p.m. All experiments were carried out in accordance with the guidelines of the "Committee for the Purpose of Control and Supervision of Experiments on Animals," (Regd.No.516/01/2019/ACPCSEA), New Delhi, India, and were approved by the Institutional Animal Ethical Committee (IAEC), Andhra University, Visakhapatnam.

Experimental design

This study employed a total of 28 animals. Animals were randomly selected and divided into four groups after one week of acclimatization, with each group consisting of seven animals (n=7). The animals in the control group (I) were fed the standard diet. Similarly, the animals in groups II, III, and IV were given haloperidol to induce catalepsy. Groups III and IV were fed FM, and the animals' body weights and food intake were recorded daily (Table 1).

The animals were evaluated behaviorally at the beginning and end of the study. The study had a total duration of 28 days. After the behavioral tests, all of the animals were euthanized with urethane anaesthesia, and their brains were quickly dissected and used for biochemical analysis.

Behavioral studies

Assessment of locomotor activity

Locomotor activity is defined as the movement and motion required to move from one location to another. The actophotometer was used to measure the locomotor activity. After 5 minutes on the actophotometer, the basal activity score of each animal was recorded. The difference in activity levels before and after FM treatment (motor activity score/5min) was measured.[13]

Assessment of motor co-ordination

The rota-rod test was used to assess motor coordination and gripping strength, as described by Hong *et al.*[14]

Assessment of catalepsy

Catalepsy in a single rat was measured stepwise using a scoring method described by Bashkatova *et al.*[15] The rat was placed on the table in step one. If the animal does not move when gently touched on the back or pushed. Step 2: The rat's front paws were alternately placed on a 3cm height block. A score was assigned if the rat did not correct its posture within 15 seconds. Step 3: Place the rat's front paws alternately on a 9 cm high block. If the rat remained in this posture for more than 15 seconds, a score for each paw was added to the step 1 and 2 scores. Thus, the highest score for an animal was 3.5 (cut-off score), which reflects total catalepsy.

Hanging wire test

The animal's grip strength was assessed using a hanging wire test. A stainless-steel wire (90cm length, 3mm diameter) placed 60cm above the ground soft surface is included. The animals were permitted to hang by their forelimbs. The latency to fall from the wire was measured, and the cut off time was recorded.[16]

Biochemical assessments

Brain tissue homogenate preparation

The animals were put to sleep using urethane anaesthesia (1.3g/kg, i.p.).[17] The skull and brain samples were dissected from the dorsal side of the skull. The cerebellum was removed, and the brains were cleaned with chilled normal saline before separating the striatum tissues from both cerebral hemispheres using the Glowinski *et al.* method.[18] The striata from both hemispheres were collected and homogenized in chilled extraction tris buffer (10 mM Tris-HCl, pH 7.4, 0.44 M sucrose, 10 mM EDTA, and 0.1% BSA) immediately after dissection. The homogenates were centrifuged for 30 minutes at 4 °C in an ice-cold (pH 7.4) extraction buffer solution. Supernatants were used for molecular and neurochemical testing.[19]

Assessment of oxidative stress markers

Lipid peroxidation assay

Cellular injury was assessed in this assay by measuring MDA levels in striatal tissue homogenate. The lipid peroxidation assay was used to assess cellular damage caused by the end product Malondialdehyde (MDA). Ohkawa *et al.*[20] described the method for estimating it.

Estimation of reduced glutathione content

GSH (mg/g) was determined using the Ellman[21] method.

Superoxide dismutase (SOD) estimation

SOD activity was determined using the Kono[22] method. The absorbance change was measured at 420nm.

Statistical analysis

All data were expressed in mean and Standard Error of Mean (S.E.M.; n=7), and differences were investigated using one-way ANOVA followed by Dunnett's test. **p 0.05 and ***p 0.001 differences were considered statistically significant.

Results

FM's effect on motor activity-related behavioral parameters

Hanging wire test

When compared to the normal control group, administration of haloperidol (1mg/kg, i.p.) for 28 days resulted in a significant decrease in time to fall by impeding grip strength ($p<0.001$). When compared to the diseased control animals, treatment with FM at both doses resulted in a significant improvement in the time to fall ($p<0.001$, $p<0.001$; Table 2).

Catalepsy by block method

When compared to the diseased control animals, haloperidol induced animals had a significant impairment in posture and motor activity (p 0.001), whereas FM low and high doses exhibited low scores and showed a significant improvement in posture and motor activity ($p<0.001$, $p<0.001$; Table 2).

Physical activity

When compared to the normal control group animals, haloperidol administration resulted in a significant decrease in locomo-

Table 1. Experimental Design. Animals were divided into 4 groups, each group consisting of seven animals (n=7).

Groups		Treatment
I.	Control	Received normal saline for 28 days
II.	Disease control	Rats induced with haloperidol (i.p.) for 28 days
III.	Treatment	Rats induced with haloperidol+low dose FM (25%)
IV.	Treatment	Rats induced with haloperidol+low dose FM (50%)

tor count ($p<0.001$). FM treatment at low and high doses significantly improved the decrease in locomotor count ($p<0.001$ and $p<0.001$, respectively; Table 2).

Rota rod test

The rotarod test assesses rodent motor coordination. When compared to the normal control group animals, haloperidol induction reduced the time to fall ($p<0.001$). When compared to the diseased control animals, the treatment groups showed significant improvement in motor coordination and increased the time taken to fall ($p<0.05$, $p<0.001$; Table 1).

Biochemical assessments

Assay for lipid peroxidation

Lipid peroxidation was significantly increased in the striatum ($p<0.001$) of diseased animals and significantly decreased in the FM treated group animals ($p<0.001$, $p<0.001$; Table 3).

SOD assay

SOD activity was significantly reduced in the striatum ($p<0.05$) when compared to the control group. FM treatment resulted in a significant increase in SOD levels in both groups ($p<0.05$, $p<0.05$; Table 3).

GSH estimation

Enzymatic antioxidant levels of GSH were significantly reduced after haloperidol administration ($p<0.001$), but significantly increased in a dose-dependent manner after FM powder treatment in both groups ($p<0.05$, $p<0.05$; Table 3).

Discussion

The current study demonstrated the anti-oxidative potential of FM in a PD model induced by haloperidol. Haloperidol has been shown in studies to reproduce a wide range of behavioral and biochemical alterations similar to those seen in PD.[3] Haloperidol, a D2 receptor antagonist used to treat agitation and aggression in the acute phase of schizophrenia, can cause extrapyramidal side effects such as akinesia and rigidity of movement.[23] Catalepsy, a bradykinetic and rigidity behavioral condition in which the animal is unable to correct externally imposed postures, can be caused by haloperidol in rodents. Haloperidol induction also blocks nigrostriatal D2 dopaminergic receptors, so it is frequently used as an animal model for the study of motor impairments and the screening of anti-parkinsonian agents.[24]

Catalepsy is a prominent feature of PD; when compared to normal control group animals, the administration of haloperidol resulted in significant behavioral changes in motor performance tests such as hanging wire, locomotor activity, rotarod test, and block method for catalepsy. When compared to disease control animals, treatment with foxtail millet powder significantly improved motor dysfunctions in all behavioral tests.

Increased oxidative stress and mitochondrial dysfunction have been linked to PD, as has nigrostriatal pathway degeneration. So, in this study, we looked at oxidative stress markers like MDA, SOD, and GSH. When comparing diseased control animals to normal control animals, we found a significant increase in MDA levels, which is a marker of lipid peroxidation and cellular injury. While the diseased control group had lower levels of the antioxidant enzymes SOD and GSH. The antioxidant levels in the fox tail millet-treated groups were significantly higher. The findings suggest that foxtail millet may have antioxidant properties.

While oxidative stress is linked to neuronal death. ROS accumulation caused by cellular redox imbalance causes neuronal injury. ROS accumulation can cause oxidative damage to lipids, proteins, DNA, and RNA, impairing neuronal function and structural integrity,[25] depending on the subcellular location of ROS synthesis.

Importantly, evidence from earlystage PD patients revealed that elevated oxidative stress is a critical feature of the early disease stages, preceding major neuron loss.[26] This implicates uncontrolled ROS production as a potential cause of dopaminergic neurodegeneration rather than a secondary response to progressive neurodegeneration.[27]

Previous research has found that FM has powerful anti-oxidative properties, as well as changes in neurotransmitter levels.[10,27] FM consumption significantly reduced kidney tissue damage in a diabetic mouse model by restoring pro-inflammatory characteristics, according to a recent study by Liu and colleagues.[28] Cooked FM consumption increased the expression of glucagon-like peptide-1 receptor (GLP-1R) and phosphoinositide-protein kinase B (p-AKT/AKT) levels in a diabetic mouse model, whereas raw FM consumption decreased the expression of stearoyl-coenzyme A desaturase 1 (SCD1) levels.[29] In physiological conditions, SCD 1 activates protein kinase B (Akt), which is involved in cell proliferation, apoptosis, and glucose

Table 2. Effect of FM on various behavioural parameters on haloperidol-induced catalepsy in rats.

Groups	Hanging wire test Time (sec)	Catalepsy block method Fall of time (score)	Locomotor activity (counts)	Rota-rod test Time (sec)
Normal Control	111±5.64	0±0.00	362.85±14.42	110.57±4.74
Disease Control	31.83±8.29***	1.92±0.16	117.5±11.62***	39.28±6.81***
FM (25%) + Haloperidol	51.85±12.34***	1.5±0.21***	250±18.80***	76.0±8.31**
FM (50%) + Haloperidol	71.28±9.20***	1.08±0.20***	315.57±13.30***	94.71±9.71***

Values were expressed as mean ± SEM (n=7). All the data were analysed by one-way ANOVA followed by Dunnet's test and significant change was reported at p<0.05, ***. **indicate p<0.001, p<0.05.

Table 3. Effect of FM on various oxidative stress parameters on haloperidol-induced catalepsy in rats.

Groups	SOD (U/g)	MDA (nmol/mg)	GSH (nmol/mg)
Normal Control	4.71±0.21	1.11±0.09	7.53±0.41
Disease Control	3.26±0.19**	2.37±0.10***	3.71±0.23***
FM (25%) + Haloperidol	3.43±0.20**	1.81±0.08***	3.54±0.29**
FM (50%) + Haloperidol	3.88±0.22**	1.43±0.14***	4.43±0.30**

Values were expressed as mean ± SEM (n=7). All the data were analysed by one-way ANOVA followed by Dunnet's test and significant change was reported at p<0.05, *** indicate p<0.001, ** p<0.05.

metabolism. FM consumption significantly reduced the proliferative potential of breast cancer cells.[30] FM inhibited inflammation in mice[31] and murine macrophages[32] by increasing the antioxidant cytokine IL-10 and blocking the nuclear factor-kappa B (NF-kB)-p65 translocation.[31] These findings suggest that foxtail millet acts as an antioxidant, reducing motor dysfunctions in haloperidol-induced catalepsy.

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