

Morphological and Histochemical Investigation of the Role (S) of *Moringa Oleifera* (Lam) on 3-Nitropropionic Acid Model of Huntington's disease

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Abstract: Aim: This study evaluated the role of *Moringa Oleifera* (MO) in the treatment of Huntington's disease induced by 3-Nitropropionic Acid (3-NP). **Material and Method:** Forty adult mice weighting between 25-32g were used and divided into four groups (A, B, C and D), Group A serves as the control (Received food and ad libitum), B (Received MO+3-NP), C (Received only 3-NP) and D (Received 3-NP + MO). 3- Nitropropionic Acid (20mg/kg) was administered to the animals intraperitoneal for 7 days. MO was administered orally (250mg/kg) for a period of 7 days. Weights of the animals were monitored periodically using the digital weighing balance. Animals were sacrificed at the end of the experiment and the brain tissue was excised for morphological and histochemical study.

Results showed cyto-architecture derangement of the brain tissue in the Huntington's group, while the preventive group showed some degree of preservation of the tissue architecture. **Conclusion:** 3-Np induced huntington's disease model in mice as expected with the characteristics of demyelination, chromatolysis, exaggerated protein clumps aggregations on the tissue organ and *Moringa oleifera* shows little curative effect on the neuronal neuro-degeneration.

Key word: huntington's disease, 3-Nitropropionic acid, *Moringa*,

I. INTRODUCTION

Huntington's disease (HD) is a genetic neurodegenerative disorder in which mental declination is observed and muscle coordination is impaired. It can also define as progressive neurodegenerative disorder associated with severe degeneration of basal ganglia neurons, (Freeman *et al.*, 1993), especially the intrinsic neurons of the striatum, and characterized by progressive dementia and involuntary abnormal choreiform movements. As broad as knowledge is in the field of Neuroscience, there has been no cure available to completely cease or reverse the progressive neurodegeneration and behavioral consequences of the HD disease (Brandt *et al.*, 1995). It is the most common genetic cause of

abnormal involuntary writhing movements called chorea, which is why the disease used to be called Huntington's chorea (Barbreau *et al.*, 1979). The disease can also be caused by an autosomal dominant mutation in either of an individual's two copies of a gene called Huntington. The Huntington gene provides the genetic information for a protein that is also called "huntington". Huntington gene results in a different form of the protein, which gradually damages cells in the brain, through mechanisms that are not fully understood. Genetic testing can be performed at any stage of development, even before the onset of symptoms. Huntington's disease HD (Brandt *et al.*, 1995). Symptoms of the disease can vary between individuals and affected members of the same family, but usually progress predictably (Cockrell *et al.*, 1995). The earliest symptoms are often subtle problems with mood or cognition. A general lack of coordination and an unsteady gait often follows (John *et al.*, 2010). As the disease advances, uncoordinated, jerky body movements become more apparent, along with a decline in mental abilities and behavioral symptoms. Physical abilities gradually worsen until coordinate becomes difficult (Brandt *et al.*, 1995). Huntington's disease shows a decrease in activity of the mitochondrial pathway, complex II-III. Such deficiencies are often associated with basal ganglia degeneration (Beal, 1998).

The Basal ganglia disease refers to a group of physical dysfunctions that occur when the group of nuclei in the brain known as the basal ganglia fails to properly suppress unwanted movements or to properly prime upper motor neuron circuits to initiate motor function. (Namara *et al.*, 2008).

In the neurodegenerative disorder of HD is the progressive loss of striatal neurons (Albin *et al.*, 1990). Although the mechanisms of selective striatal damage in HD are not known, the activation of excitatory amino acid receptors have been

implicated (Beal, 1998). In addition, various toxins have been found to cause striatal lesions reminiscent of the neurochemical and anatomical changes associated with this disorder (Beal, 1998). One of such toxin is 3-nitropropionic acid 3-NP., a naturally occurring plant mycotoxin that is an irreversible inhibitor of succinate dehydrogenase, a subunit of complex II of the electron transport chain and a component of the Krebs's cycle (Beal, 1998; Borlong, 1997; Cheng, 1994). It is not known if 3-NP results in oxidative stress in brain regions other than striatum. It is also not known if the oxidative stress precedes or follows striatal lesions induced by 3-NP. The disease can affect both men and women.

3-nitropropionic acid (3-NP), an inhibitor of the mitochondrial citric acid cycle, results in a progressive locomotor deterioration resembling that of HD. 3-NP produces very selective striatal degeneration. It differs mechanistically from excitotoxic lesions in that 3-NP irreversibly inhibits the mitochondrial citric acid cycle and leads to depressed ATP levels and elevated lactate concentrations. Recent neurochemical studies have implicated lowered glutamate levels and impaired oxidative energy metabolism as underlying mechanisms for many neurodegenerative disorders, including HD. Because of the mechanistic and pathologic similarities between 3-NP lesions and HD, 3-NP has been proposed as an alternative HD model. 3-NP injections leads to sustained hyperactivity (early HD) or hypoactivity (late HD) (Duffy, 2013).

The 3-NP closely resembles that of HD. This body of evidence suggests that the 3-NP model is an improved HD model and may offer a unique system wherein testing of experimental treatments for HD can be carried out across different stages of the disease. 3-NP model will be very useful especially in assessing the efficacy of treatment modalities, e.g. neural transplantation, during the progression of the disease.

Moringa oleifera has been found as a potent anticancer plant and several bioactive compounds with significant antitumor activity have been discovered from *Moringa*. Among bioactive compounds from *Moringa*, niazimicin, a *Moringa oleifera* leaves thiocarbamate was found to have potent anticancer activity (Guevara *et al.*, 1999). Beside leaves, *Moringa* seed extracts also have anticancer activity through its effects on hepatic carcinogen metabolizing enzymes, and antioxidant property (Bharali *et al.*, 2003). *Moringa* leaves and *moringa* pods are a nutritional powerhouse and provide a great range and amount of essential proteins, vitamins and minerals. *Moringa* is a rich source of essential amino acids, which are the building blocks of proteins. It also contains a significant amount of vitamins such as vitamin A, vitamin B1 (thiamine), B2 (riboflavin), B3 (niacin), B-6, folate and ascorbic acid (vitamin C). (Bharali *et al.*, 2003). The mineral wealth of *moringa* includes calcium, potassium, iron, magnesium, phosphorous and zinc. It contains very low amount of fats and offers no harmful cholesterol.

II. MATERIALS AND METHOD

40 Male and Female Mice, 3-Nitropropionic acid, cotton wool, dissecting set, dissecting board, EDTA bottles, specimen bottles feeding cans, Methylated spirit, Needle and Syringe, oral cannula, water, weighing scale, sensitive balance.

Plant Extraction Procurement and Administration.

Leaves were plucked, taken to a taxonomist at the Department of Botany, University of Ibadan for authentication after which a voucher specimen was deposited at Ibadan Herbarium with a reference number 110265. The plant was first soaked in ethanol, air-dried and grounded to powder using an electric grinder and then dissolved in distilled water to get desired aqueous extract. The extract was filtered and the filtrate was concentrated at 30^o C using the vacuum rotary evaporator to completely remove the water. The aqueous extract of *Moringa oleifera* (AMO) was stored in a desiccators until used. The dosage was administered in mg/kg of the body weight orally with the aid of suitable oro-gastric tube daily.

Induction of Drug

The 3-Nitropropionic Acid was given based on the body weight of the animals and the route of administration was through intra peritoneal region with standard dose of 20mg/kg (Micheal., 1999). The extract of leaves of MO was given orally at the standard dose of 250mg /kg body weight for 7 days (between 10:00 and 11:00 am) after which they were sacrificed. The dose was standardized in the laboratory.

Animal Care and Management

The study was performed using forty male and female adult mice weighing (25 - 30g), the animals were housed in standard laboratory cages, Faculty of Basic Medical Science Olabisi Onabanjo University Ogun state. They were fed and acclimatized for two weeks before the commencement of experimental protocol.

The mice were divided into 4 groups, of 10 mice each having equal number of both sexes; the body weight of the mice was measured periodically throughout the study. (Fawcett, 2012)

Experimental Design

Grouping of animals: The animals were divided into 4 groups:

Group A. Control group received distilled water and food ad libitum.

Group B. Were given AMO for 7 days + 3-nitropropionic acid for another 7 days [pretreated group]. (AMO+ N)

Group C. Were given 20 mg/kg 3-nitropropionic acid (N) for 7 days

Group D. Was given 3-nitropropionic acid for 7 days + AMO for another 7 days.[post treated group]

(N+ AMO)

Animal sacrifice

The animals were sacrificed by cervical dislocation. The skull of the mice were dissected and the brain excised and placed in a petri-dish coronal sections of the basal ganglia were carefully cut out, homogenized and centrifuged and the clear supernatant was collected into plain bottles for quantitative histochemical studies or assays.

Other sections of the basal ganglia were fixed in 10% formol calcium for histological studies.

Histological processing

- Haematoxyline and Eosin for generalhistoachitecture
- Cresyl Fast Violet for Nisslsubstance
- Luxol Fast Technique for Myelin

III. RESULTS

Relative Brain Weight Male Mice

The group D that took *moringa oleifera* after taken 3-Nitropropionic acid had highest Relative Brain Weight [RBW] while there is increase in the treated group when compared with the control group. These differences were statistically not significant ($P \leq 0.05$).

Relative Brain Weight of Female Mice

The group D that took after taken 3-Nitropropionic acid had highest Relative Brain Weight [RBW] than the Control. These differences were not statistically significant ($P \geq 0.05$).

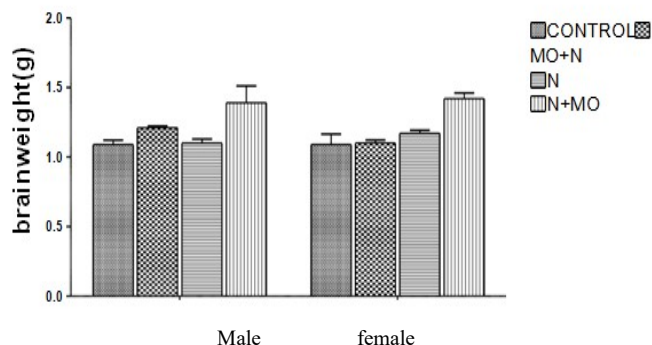


Figure 1: Relative Brain Weight of both Male and Female mice

KEY

CONT—CONTROL

MO--MORINGA OLEIFERA

N-----3-NITROPROPIONICACID

Relative Brain Weight (Male and Female) Mice

The table below showed the relative brain weight of the experimental Mice in all groups at the end of the experiment, the relative brain weight varied, the group D female had the highest brain weight than that of male, the weight loss is comparatively more in male than female groups. These differences were not statistically significant ($P \geq 0.05$).

Figure 2: Relative Brain Weight of male and female

	Control	MO + N	N	N + MO
Male	1.09 ± 0.07	1.21 ± 0.03	1.10 ± 0.05	1.39 ± 0.21
Female	1.09 ± 0.13	1.10 ± 0.05	1.17 ± 0.05	1.42 ± 0.09

4.1Photomicrographs of control groups stain with H & E (male and female) showing normal neurons (Green arrows) and blood vessel (blue arrow) and protein clumps (Red arrow)

PLATE 4.1: Photomicrographs of Control mice Striatum (a)Male H&E X100; (B) Female H&E X100

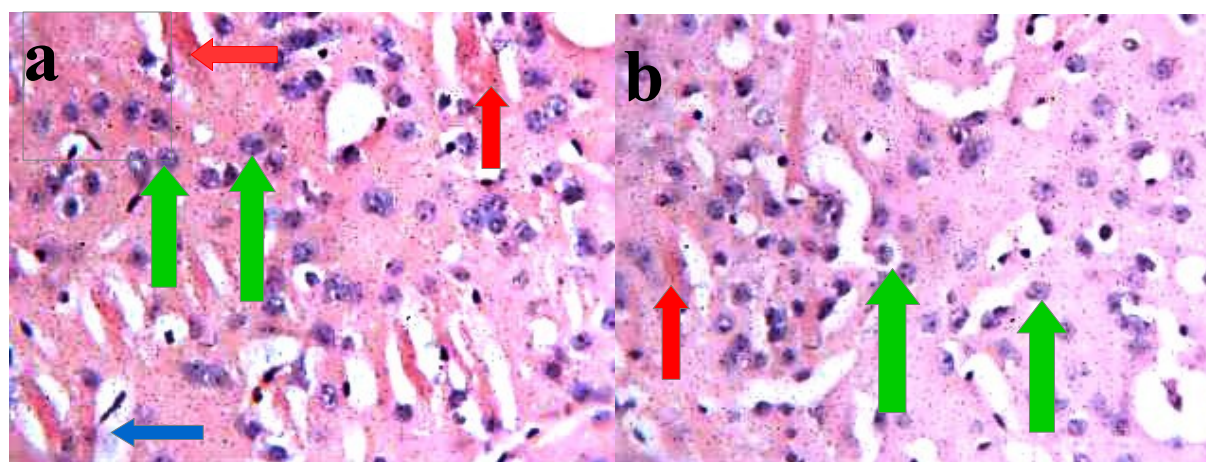


PLATE 4.1: Photomicrographs of Control mice Striatum (a₁)Male H&E X400; (b₁) Female H&E X400

Plate 4.1.2 : Photomicrographs of group B (C= male, D= female) that took Moringa and 3- nitropropionic acid (Mag; X400). Neuronal degeneration (yellow arrow), protein clumps (Red arrow) normal neurons (Green arrow)

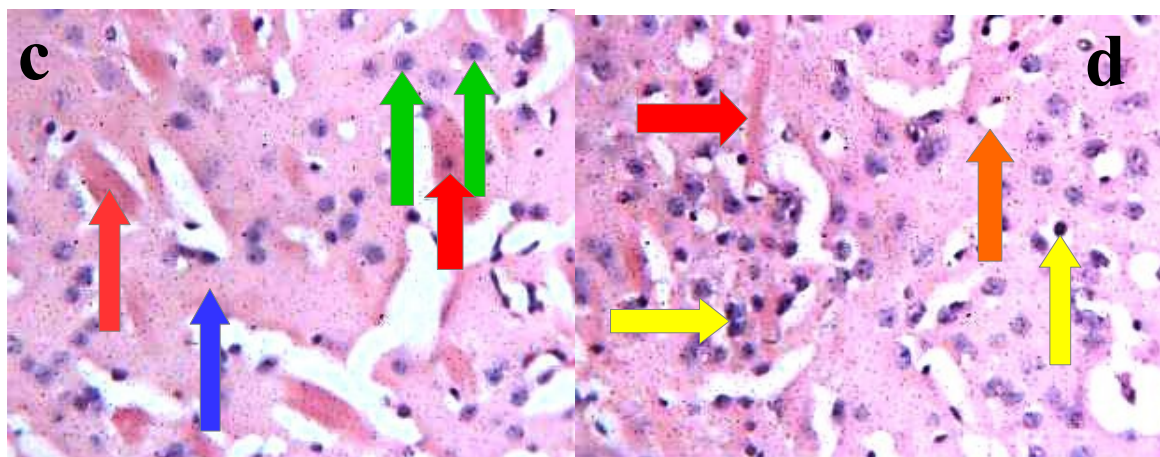


PLATE 4.1.2: Photomicrographs of MO+N Striatum (c)Male H&E X400; (d) Female H&E X400

Plate 4.1.3: Photomicrographs of group C (E=male and F=female), showing serious cellular atrophy and neuronal degeneration characterised by pyknotic nuclei (yellow arrows), protein clumps (Red arrow)

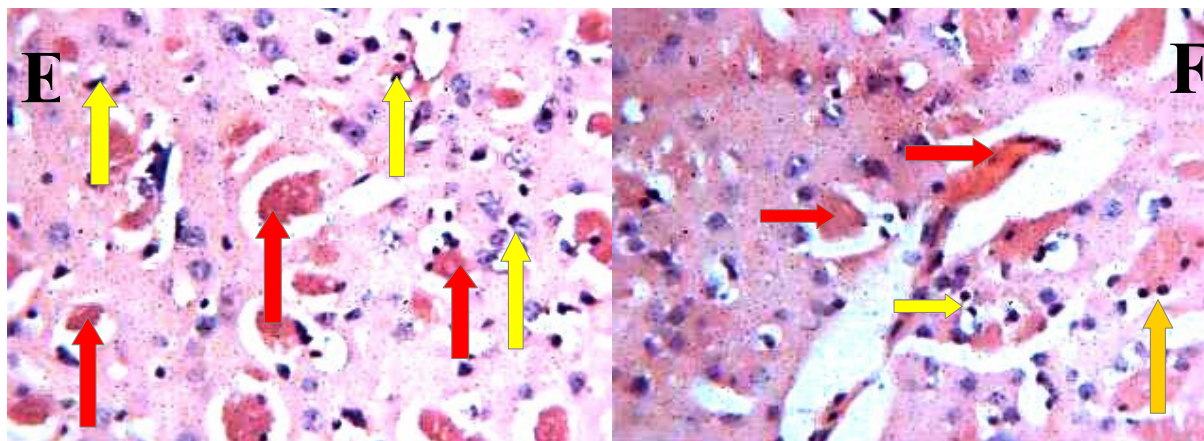


PLATE 4.1.3: Photomicrographs of (N)mice Striatum (g)Male H&E X400; (h) Female H&E X400

Plate 4.1.4: Photomicrographs of group D (N+MO) (G =Male and H= female) treated with 3- nitropropionic acid and Moringa oleifera. In this plate, we observed protein clumps which is prominent in female compared to the male (Red arrow) Neuronal nuclei (Green arrow)

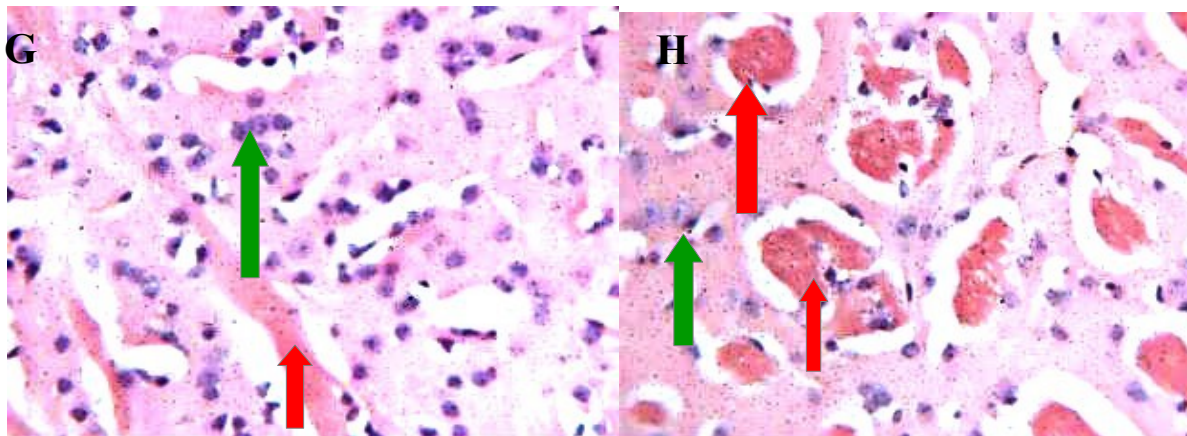


PLATE 4.1.4: Photomicrographs of N+MO Striatum (i)Male H&E X400; (j) Female H&E X400

Plate 4.2.1: Photomicrographs of control group (I =male and j =female) stain with cresy fast violet stain for nissl substance. Nissl substance are showed by the dark blue stain in the neuron pointed to by the bluearrows

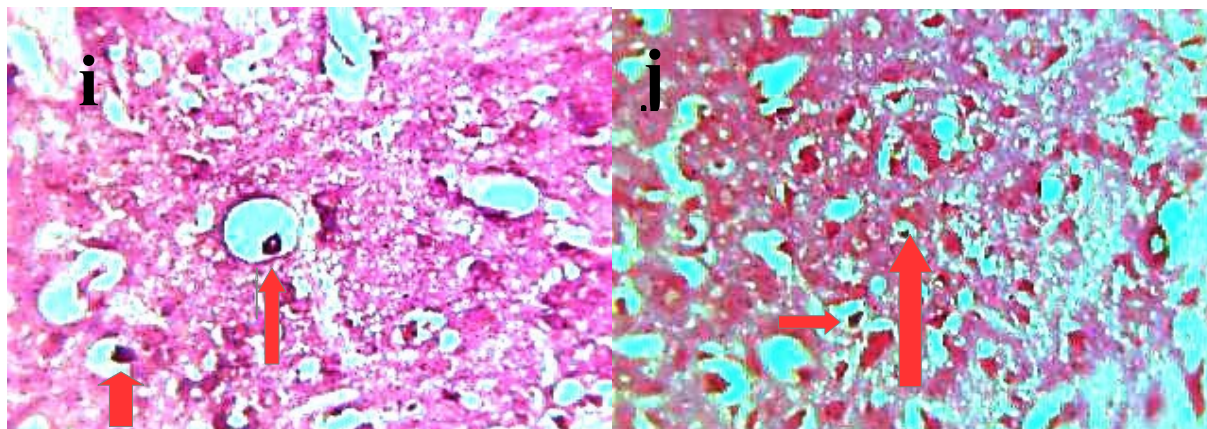


PLATE 4.2.1: Photomicrographs of Control mice Striatum (a)Male Cresty fast X400; (B) Female H&E X400

Plate 4.2.2: Photomicrographs of group B(MO+N) treated with *Moringa oleifera* and 3-nitro-propionic acid, stained with Cresyl Fast Violet. Loss of nissl substance was so obvious (Red arrows) as well as some blood vessels (Green arrows) most especially in group B male.

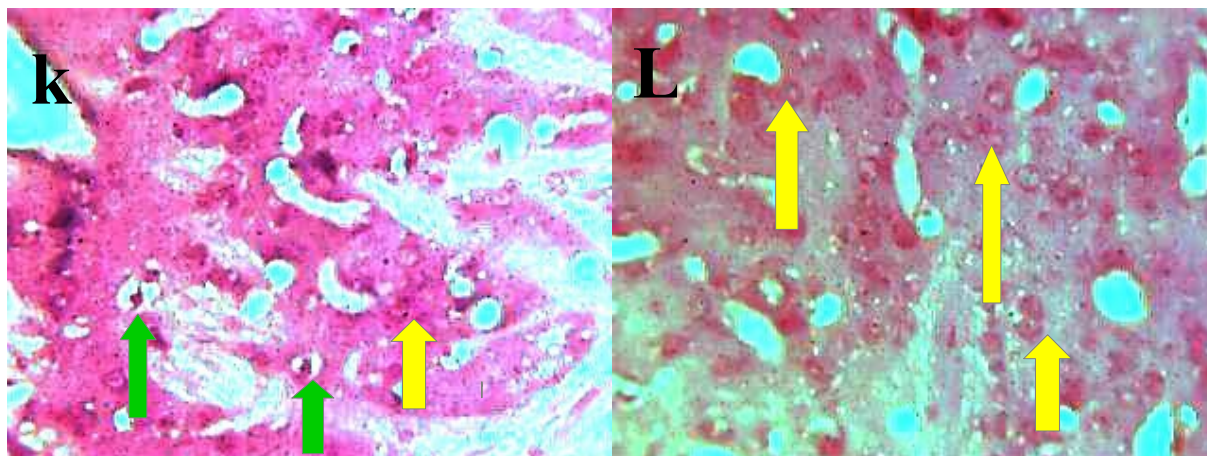


PLATE4.2.2: Photomicrographs of MO + N mice Striatum (k) Male Cresty fast X400; (L) Female cresty fast X400

Plate 4.2.3: Photomicrographs of group C (N) treated with only 3-nitro-propionic acid and stained with Cresyl Fast Violet. Neuronal degeneration along with loss of nissl substance was observed and few blood vessels (Green arrow).

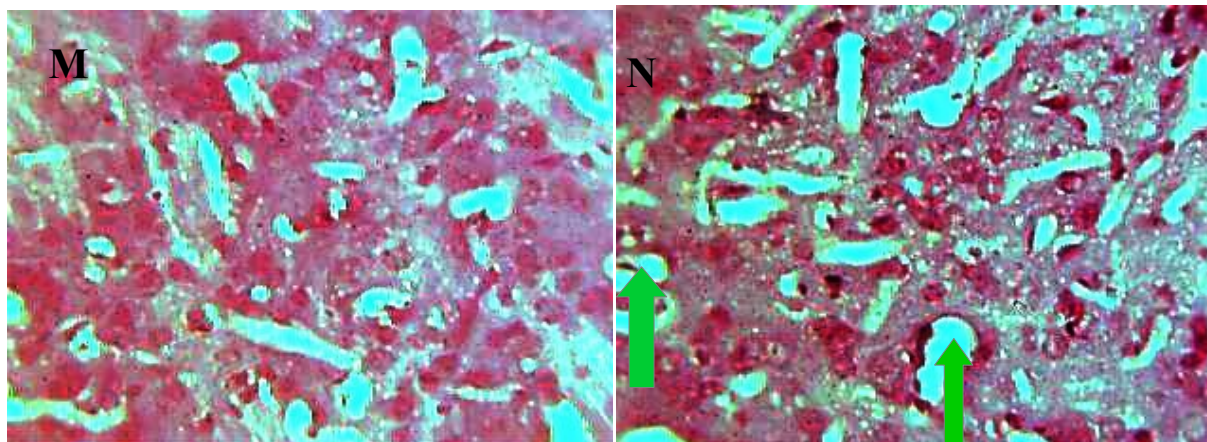


PLATE4.2.3: Photomicrographs of N mice Striatum (M)Male Cresty fast X400; (N) Female cresty fast X400

Plate 4.2.4: Photomicrographs of group D (N+MO)treated with 3-nitro-propionic acid and Moringa then stained with Cresyl Fast Violet. Showing loss of Nissl substance (Red arrow), blood vessels (Green arrow) and slight Nissl substance (Yellow arrow)

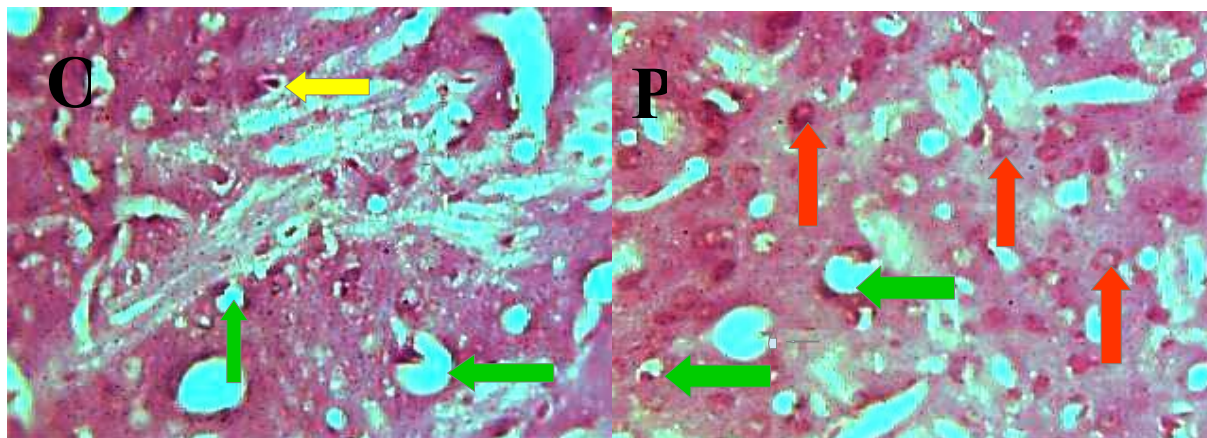


PLATE4.2.4: Photomicrographs of N+MO mice Striatum (O)Male Cresty fast X400; (P) Female cresty fast X400

Plate 4.3.1: Photomicrographs of Control groups (T=male and S=female) stained with Luxol Fast Blue (LFB). Red arrows showing myelin sheath.

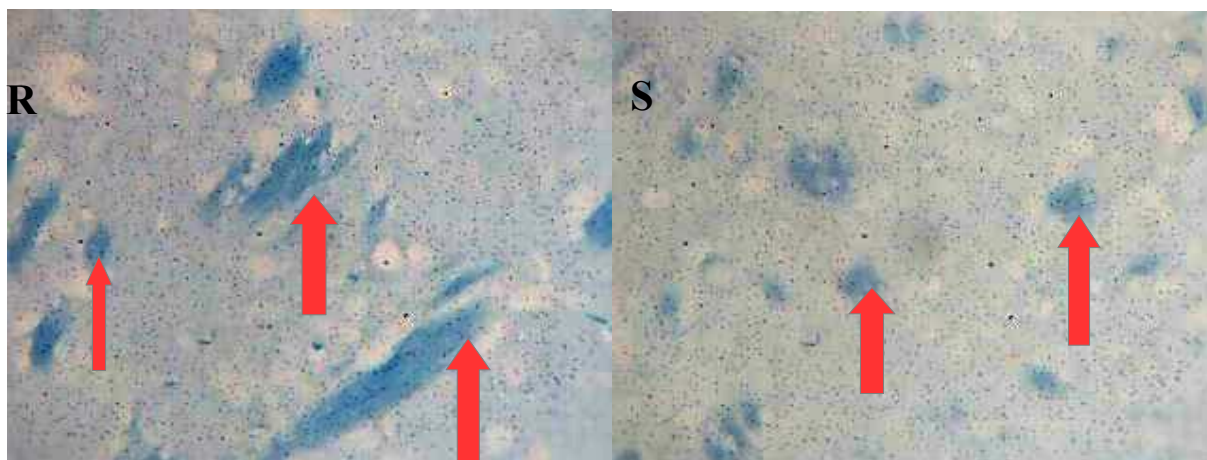


PLATE4.3.1: Photomicrographs of control mice Striatum (R)Male luxol fast X400; (S) Female luxol fast X4

Plate4.3.2: Photomicrographs of group B (MO+N)(T=male and U=female) treated with Moringa *Oleifera* and 3-Nitro-propionic acid and stained with Luxol Fast Blue. Showing normal myelin sheath (Red arrow)

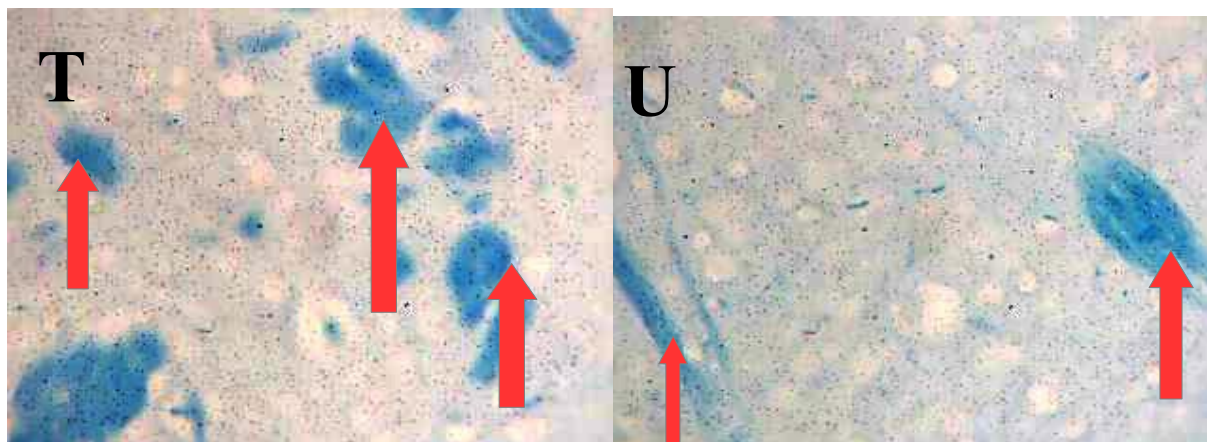


PLATE4.3.2: Photomicrographs of MO+N mice Striatum (T)Male luxol fast X400; (U) Female luxol fast X400

Plate 4.3.3: Photomicrographs of group C (N) (W=male and X=female) treated with only 3-Nitro-propionic acid and stained with Luxol Fast Blue. Showing slight demyelination (Yellow arrow) normal myelin sheath (Red arrow).

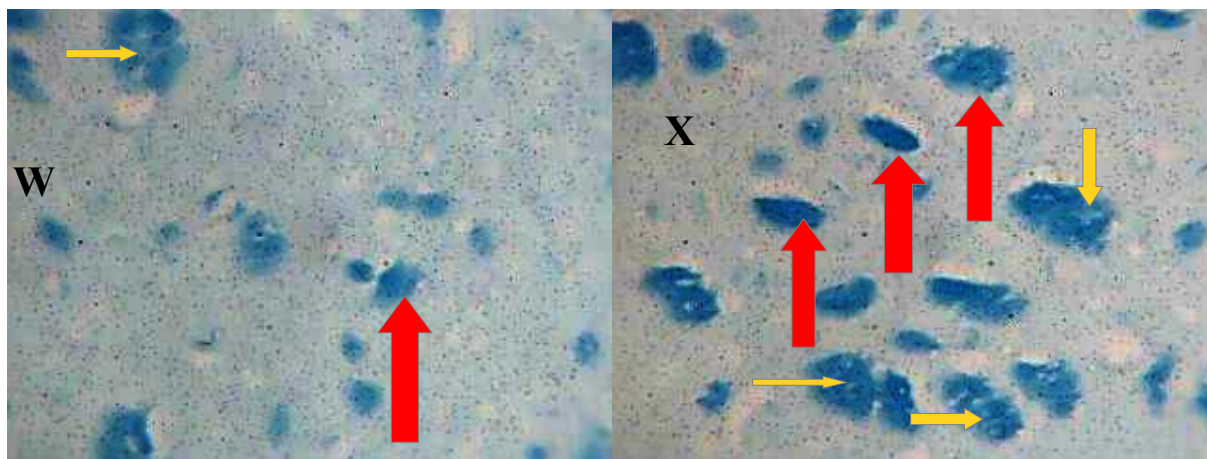


PLATE4.3.3: Photomicrographs of (N) mice Striatum (W)Male Luxol fast X400; (X) Female luxol fast X400

Plate 4.3.4: Photomicrographs of group D (male and female) treated with 3-Nitro-propionic acid and *Moringa oleifera* then stained with Luxol Fast Blue. Showing normal myelin sheath (Red arrow); slight demyelination (Yellow arrow).

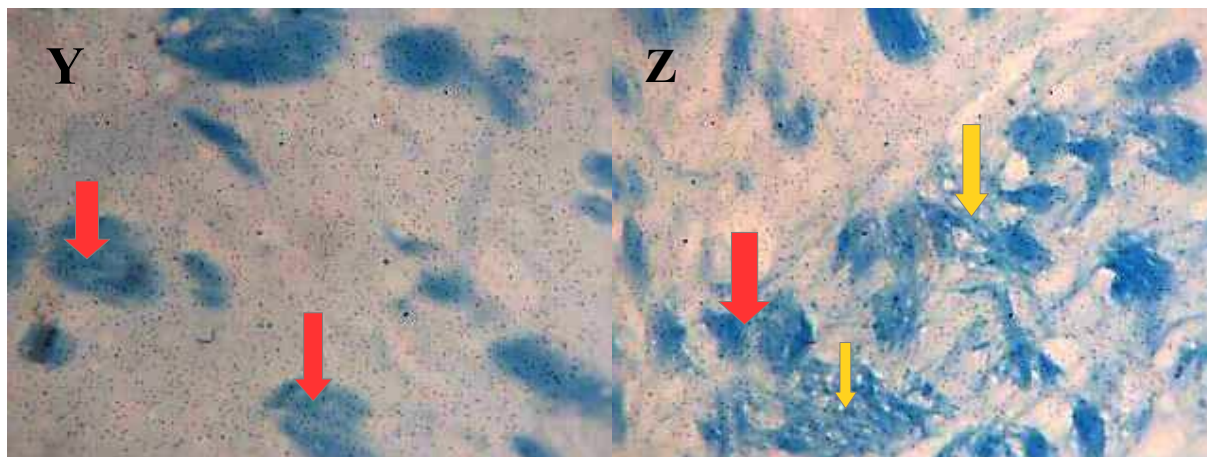


PLATE4.3.4: Photomicrographs of MO+N mice Striatum (Y)Male Luxol fast X400; (Z) Female luxol fast X400

IV. DISCUSSION

In this study we explored the role of *moringa oleifera* on 3-Nitropropionic acid induced Huntington disease. Histological and Morphological findings showed that Huntington disease resulted generally in neuronal toxicity which resulted in neuronal degeneration and protein clump, also notice that neurons in male are lesser than that of the female groups. Group C (that took only 3-NP for seven days) shows increased in protein clumps and increased in neuronal degeneration caused by pyknotic and serious cellular atrophy when compared with preventive group which is group B which shows that *moringa oleifera* as little effect on the neuronal neuro-degeneration. Group D (That took 3-Np for seven days and *Moringa oleifera* for another seven days) shows prominent protein clumps in female group than the male group. Neuronal cell death lead to apparent reduction in neuronal density while *Moringa* does little to prevent or reversed this trend. HD affects the whole brain, but certain areas are more vulnerable

than others. The most prominent early effects are in a part of the basal ganglia called the neostriatum, which is composed of the caudate nucleus and putamen (Walker, 2007), these areas are affected according to their structure and the types of neurons they contain, reducing in size as they lose cells (Walker, 2007). Striatal spiny neurons are the most vulnerable, particularly ones with projections towards the external globus pallidus, with interneurons and spiny cells projecting to the internal pallidum being less affected. HD also causes an abnormal increase in astrocytes and activation of the brain's immune cells, microglia (Duffy, 2013).

Our histochemical results using Luxol fast blue showed slight demyelination especially in the larger bundles in the Huntington's induced rats, whereas *Moringa Oleifera* appeared to boost myelination. Myelination is very important in measuring the quality of synapses and possible communicational relationships between neurons. It provides information on the integrity of cellular communication; it

could also provide information on possible degeneration processes. Axonal degeneration is a significant determination of neurological compromise. The myelin of particularly heavily myelinated axons breaks down prematurely in HD patients. One explanation for this is that axons with thicker myelin sheaths depend more heavily on myelin basic protein—an important protein in myelin that helps to maintain the structure of myelin—than axons with thinner myelin sheaths do. Production of myelin basic protein is normally supported by brain-derived neurotrophic factor (BDNF), whose production is supported by the normal huntington protein. Production of BDNF decreases drastically as a result of mutant huntington

Cytology architectural of Nissl integrity was observed using cresyl fast violet staining. This stain gives a better understanding of cytological conditions; which revealed neuronal degeneration, increase in the number of glia, Pyknosis and chromatolysis were seen in the treated groups relative to the control group, loss of nissl substance, and blood vessels were observed in treated group B and D while group C that took only the drug shows neuronal degeneration than that of the preventive group and curative group. The demonstration of Nissl substance neuron helps to appreciate the functional integrity of the cells; thus, demonstration of the Nissl substance as well as its intensity gives useful information about the tissue structural and functional integrity.

Relative Brain Weight [RBW] is the ration of the brain size or weight to the whole organism body (Perpelkina *et al.*, 2013) It is important in the measure of brain development and function. Relative Brain weight was seen to decrease in the treated groups relative to the control except in group D (N+MO) which had its relative brain weight increased. It can be said from the result that has ameliorative effects on 3-Nitropropionic acid induced Huntington disease as supported by Kumar, 2003 that provides a rare combination of zeatin (a potent antioxidant), quercetin (a flavonoid known for its ability to neutralize free radicals and relieve inflammation), beta-sitosterol (a nutrient superstar that blocks cholesterol formation or build-up and is an anti-inflammatory agent for the body), caffeoylquinic acid (another powerful anti-inflammatory compound), and kaempferol (a key nutrient that promotes healthy body cellular function). (Kumar and Kalonia, 2010).

Body weight of the animal were measure periodically, increase in the mean body weights of the experimental mice were uniform throughout the period of acclimatization for all groups. There was a decrease in the mean bodyweight of all experimental animals (in the preventive, toxic and curative groups) at the end of the second week induction period; the decrease was statistically significant when compared with the control. It could therefore be asserted that the decrease in the body weight were caused by induction of 3-NP which could be attributed to the reduction of food intake by the inducted animals.

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