Nuerobehavioural Investigation of The Role/S of Moringa *Oleifera* On 3-Nitropropionic Acid Model of Huntington's Disease

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Abstract:

Background: Huntington's disease (HD) is a neurodegenerative disorder characterized by motor impairment that contribute to the death of striatial neurons and psychiatric symptoms. Systemic administration of 3-Nitropropionic Acid (3-NP) cause selective striatial degeneration similar to that seen in HD. Recent studies clearly demonstrate that increase oxidative stress is one of the major deleterious even in the 3-NP-induced neurodegenerative process. Moringa *oleifera* (MO) has been studied for the anti-oxidant, anti-inflammatory properties. This study evaluated the Neurobehavioral role of MO following 3-NP model of Huntington's disease.

Methodology: Thirty-two 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 for 7 days) were induced intraperitoneal to the animals. MO were administer orally (250mg/kg) for a period of 7 days. Weights of the animals were checked periodically using the Acucuscope weighing Balance. Neurobehavioral assessment was carried out after administration. Animals were sacrificed at the end of the experiment Neurotransmitters- Dopamine and Glutamate were assayed in tissue homogenates and the levels were quantified.

Results showed increased tissue levels of glutamate and dopamine and impaired neurobehavioural parameters in the Huntington's group, while the preventive group showed some degree of preservation of the normal parameters.

Conclusion: 3-Np induced huntington's disease model in mice as expected with the characteristics of increased tissue levels of glutamate and dopamine and impaired behavioural and locomotive indices.

Key words: **huntington's disease, 3-Nitropropionic acid, Moringa, Neurobehavioral & Neurotransmitter**

I. INTRODUCTION

Huntington's Disease(HD) is a neurodegenerative genetic disorder that affects muscle coordination and leads to mental decline and behavioral symptoms or can also define as progressive neurodegenerative disorder associated with severe degeneration of basal ganglia neurons, (14),especially the intrinsic neurons of the striatum, and characterized by progressive dementia and involuntary abnormal choreiform movements. Despite our increasing knowledge of the pathophysiology of HD, culminating with the discovery of the gene underlying HD, there has been no cure available to completely cease or reverse the progressive neurodegeneration and behavioral consequences of the disease (6). 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.(2). The disease can also be caused by an autosomal dominant mutation in either of an individual's two copies of a gene called Huntingtin. The Huntingtin gene provides the genetic information for a protein that is also called "huntingtin". Huntingtingene 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 (6). Symptoms of the disease can vary between individuals and affected members of the same family, but usually progress predictably (10). The earliest symptoms are often subtle problems with mood or cognition. A general lack of coordination and an unsteady gait often follows. (17). 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 coordinated movement becomes difficult (6). Mental abilities generally decline into dementia. Huntington's disease shows a decrease in activity of the mitochondrial pathway, complex II-III. Such deficiencies are often associated with basal ganglia degeneration (3).

The basal ganglia are a collective group of structures in the brain. These include the striatum, (composed of the putamen and caudate nucleus), globus pallidus, substantia nigra, and the subthalamic nucleus It is important to note, however, that the striatum and globus pallidus may be considered anatomically distinct from the substantia nigra, nucleus accumbens, and subthalamic nucleus. 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. (19).

In the neurodegenerative disorder of HD is the progressive loss of striatal neurons (1).Although the mechanisms of selective striatal damage in HD are not known, the activation of excitatory amino acid receptors have been implicated (3). In addition, various toxins have been found to cause striatal lesions reminiscent of the neurochemical and anatomical changes associated with this disorder (3). 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 Kreb's cycle (3, 5,11). 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)(20).

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 **(MO)** 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 (15). Beside leaves, Moringa seed extracts also have anticancer activity through its effects on hepatic carcinogen metabolizing enzymes, and antioxidant property (4). 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

30 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, barnes mazes and open field mazes.

2.1 Plant Extraction Procurement Of 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 olifera* (MO) 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.

2.2 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.

2.3 Animal Care and Management

The study was performed using forty male and female adult mice weighing (25 - 30g), the animals were house in standard laboratory cages, Faculty of Basic Medical Science Olabisi Onabanjo University Ogun state. They were fed and acclimatize 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 measure periodically throughout the study and the animals are subjected to neurobehavioral procedures before the induction and after the experimental protocol (13)

2.4 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 MO for 7 days + 3-nitropropionic acid for another 7 days [pretreated group].(MO+ 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 + MO for another 7 days.{post treated group}(N+ MO)

2.5 Open Field Procedure

Open field is use to determining anxiety test for the assessment of anxiety and locomotor activity in Mice.

2.5.1 Apparatus

The open field apparatus was constructed of white plywood and measured 72 x 72 cm with 36 cm walls. One of the walls was clear Plexiglas, so mice could be visible in the 2 apparatus. Blue lines were drawn on the floor with a marker and were visible through the clear Plexiglas floor. The lines divided the floor into sixteen 18 x 18 cm squares. A central square (18 cm x 18 cm) was drawn in the middle of the open field (8). The central square is used because some mouse strains have high locomotor activity and cross the lines of the test chamber many times during a test session. Also, the central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations (9).

2.5.2 Procedure

Mice were carried to the test room in their home cages and were handled by the base of their tails at all times. Mice were placed into the center or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5 minute test, mice were returned in their home cages and the open field was cleaned with 70 % ethyl alcohol and permitted to dry between tests. To assess the process of habituation to the novelty of the arena, mice were exposed to the apparatus for 5 minutes on 2 consecutive days.

2.6 Behaviours Scored

The behaviours scored (8) included:

1. Line Crossing: Frequency with which the mice crossed one of the grid lines with all four paws.
2. Center Square Entries: Frequency with which the mice crossed one of the red lines with all four paws into the central square.
3. Center Square Duration: Duration of time the mice spent in the central square.
4. Rearing: Frequency with which the mice stood on their hind legs in the maze.
5. Stretch Attend Postures: Frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
6. Grooming: Duration of time the animal spent licking or scratching itself while stationary.
7. Freezing: Duration with which the mouse was completely stationary.
8. Urination: number of puddles or streaks of urine.
9. Defecation: number of fecal boil produced.

2.7 Barnes Maze

The Barnes maze is a visuo-spatial learning and memory test originally designed for use with rats, and later adapted for use with mice. The Barnes maze design and test procedure vary across studies using mice, Barnes maze design and test procedure on learning and memory in mice have not yet been investigated (12)

Therefore the present experiment investigates whether test procedures, such as the number of habituation trials and parameters of the probe trial (correct zone size and trial length) influence learning and memory performance on three.

2.7.1 Barnes Maze Procedure

Barnes maze was administered to assess cognitive deficits in learning and memory of 3×Tg mice compared to the WT group. The maze was made from a circular, 13-mm thick, white PVC slab with a diameter of 48". Twenty holes with a diameter of 1.75" were made on the perimeter at a distance of 1" from the edge. This circular platform was then mounted on top of a rotating stool, 35" above the ground and balanced.

After testing each mouse, the cleaning of the quadrant of the maze around the target hole was alternated with cleaning the whole maze, using 70% ethanol.

The animals interacted with the Barnes maze in three phases: habituation (1 day), training (2–4), training 2(5-6) days in the short or long training paradigm and probe (1 day). Before starting each experiment, mice were acclimated to the testing room for 1 h. Then all mice from each cage were placed in individual holding cages where they remained until the end of their testing sessions. Holding cages were used during the experiment to control for potential artifacts that could result from housing, and remained. Additionally, using holding cages prevented potential influence by mice that had already completed the test on the mice waiting for their turn. After all mice from one home cage completed testing for the day, they were placed back in their home cage together, the holding cages were cleaned, and the next set of mice was separated into individual holding cages.

2.8 Habituation

On the habituation day, the mice were placed in the center of the maze underneath a clear 3,500-ml glass beaker for 30 s while white noise was played through a sound system. Then, the mice were guided slowly by moving the glass beaker, over 10–15 s to the target hole that leads to the escape cage. The mice were then given 2 min to independently enter through the target hole into the escape cage. If they did not enter on their own during that time, they were nudged with the beaker to enter. Getting the mice to enter the escape cages is key in "showing" them that the escape cage exists and gives them

practice in stepping down to the platform in the cage. The mice were allowed to stay in the escape cage for 1 min before being returned to the holding cage. Once all animals had completed the 1-session habituation, they were all returned to their home cage.

2.9 Trainng Procedure

In the training phase, mice were placed inside an opaque cardboard cylinder, 10" tall and 7" in diameter, in the center of the Barnes maze for 15 s. This allowed the mice to be facing a random direction when the cylinder was lifted and the trial began. At the end of the holding period, a buzzer was turned on, the cylinder was removed, and the mice were allowed to explore the maze for 2 min. If a mouse found the target hole and entered the escape cage during that time, the end-point of the trial, it was allowed to stay in the escape cage for 1 min before being returned to the holding cage. If it did not find the target hole, the mouse was guided to the escape hole using the glass beaker and allowed to enter the escape cage independently. If it did not enter the escape cage within 2 min, it was nudged with the beaker until it did. If a mouse still did not enter the escape cage after 2 min of nudging, it was picked up and manually put on the platform in the escape cage. Then it was allowed 1 min inside the escape cage before being returned to the holding cage. The buzzer was turned off once the mouse entered the escape cage. The total number of trials used was 5 for short training, 3 trials on training day 1 and 2 trials on training day 2.

During the training phase things that were noted are:

Measures of primary latency, primary HS were recorded. During the trials phase, measures of time spent per quadrant and HS per quadrant were recorded. For these analyses, the maze was divided into quadrants consisting of 5 holes with the target hole in the center of the target quadrant. The other quadrants going clockwise from the target quadrant were labeled: positive, opposite, and negative.

Primary latency was defined as the time to identify the target hole the first time, as mice did not always enter the hole upon first identifying it. **HS** was defined as nose pokes and head deflections over any hole.

Primary HS was defined as the HS before identifying the target hole for the first time. Parameters were assessed by blinded observers. About 70% of the measures were randomly reassessed by a second blinded observer to identify potential inaccuracies. Differences between the two observers were insignificant in all cases. In all the cases in which two observers scored the raw data, their scores were averaged.

2.10 Probe Procedure

On the probe day, 48 h after the last training day, the escape cage was removed, mice were placed inside the opaque cylinder in the center of the maze for 15 s, the buzzer was turned on and the cylinder removed. Each mouse was given 2 min to explore the maze, at the end of which, the buzzer was turned off and the mouse was returned to its holding cage. During the probe phase, measures of time spent per quadrant and HS per quadrant were recorded. For these analyses, the maze was divided into quadrants consisting of 5 holes with the target hole in the center of the target quadrant. The other quadrants going clockwise from the target quadrant were labeled: positive, opposite, and negative (21).

2.11 Neurotransmitter

Procedure: Two grams of the homogenised sample was weighed in digestion tubes and one tablet of the selenium catalyst was added into the tube. Ten milliliters of concentrated perchloric acid and concentrated nitric acid were added in the ratio of 1:1, the tubes were then placed in the digestion block and allowed to digest slowly. The digest was washed in a 1000 ml volumetric flask and madeup with distilled water. The washed samples were read with an Atomic Absorption Spectrophotometer using their respective lamp and wavelength for Dopamine 430 nM and Glutamate 470 nM. (22)

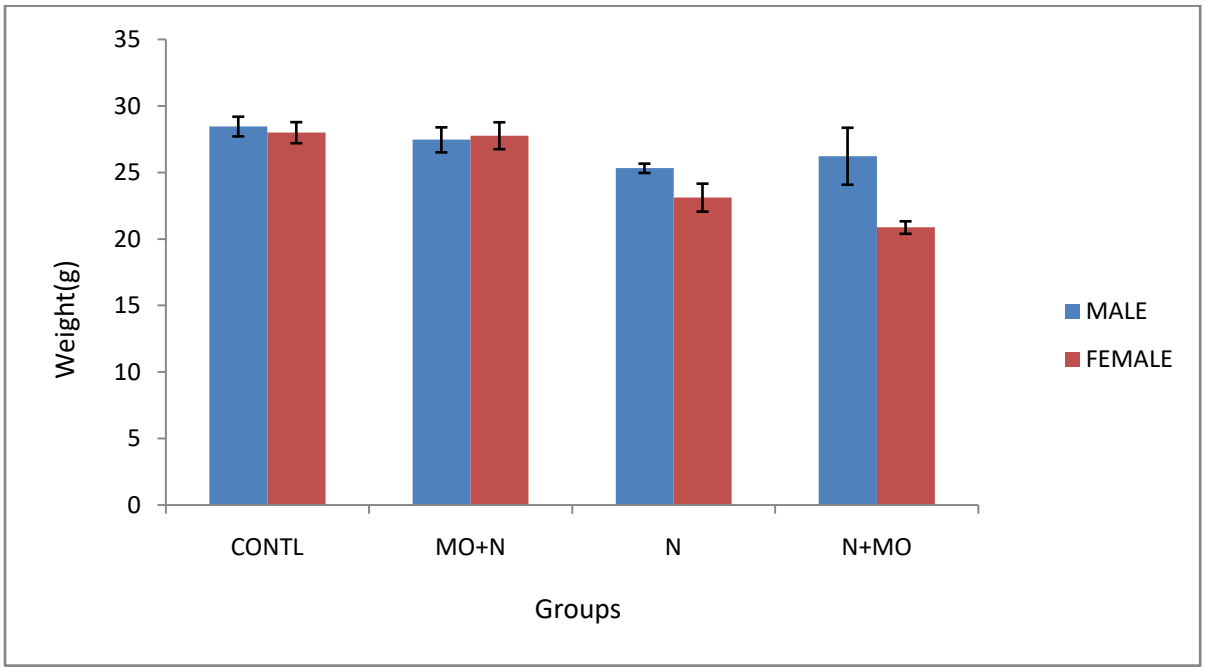
III. RESULT

Body Weight of the Male and Female

At the end of the experiment there were reduction in the weight of animals and that of the male was greater than that of the female. The control group had the highest weight gained. The group that took the 3-Nitropropionic acid before *moringa oleifera* in the female group had the lowest differences between the control and experimental groups where statistically significant.

TABLE 4.1 THE TABLE BELOW SHOWING THE RESULTS OF BODY WEIGHT IN EXPERIMENTAL GROUPS

MEAN±SEM	CONTROL	MO+ N	N	N +MO
MALE	28.46±0.74	27.46±0.94	25.32±0.35	26.23±2.14
FEMALE	28.00±0.79	27.77±1.01	23.12±1.05	20.87±0.47



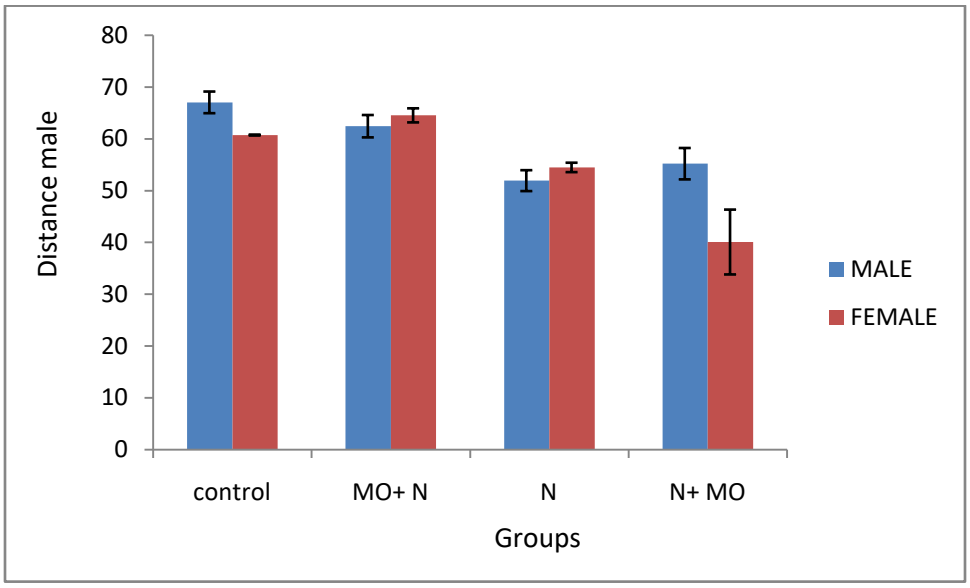
TEST DETAILS (F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	-5.70	0.74	YES	0.02
Control--- N	-1.05	1.01	NO	0.36
Control—N +Mo	1.19	0.91	NO	0.26
Mo + N---N	4.65	1.39	YES	0.02
Mo + N---N+ Mo	6.89	1.35	YES	0.00
N --- N +MO	2.24	1.16	NO	0.12

TEST DETAILS(M)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	-5.30	0.77	YES	0.02
Control--- N	-3.16	0.76	YES	0.01
Control—N +Mo	-4.07	1.43	YES	0.04
Mo + N---N	2.14	1.01	NO	0.10
Mo + N---N+ Mo	1.22	1.72	NO	0.51
N --- N +MO	-0.91	2.08	NO	0.68

Distance covered by male and female mice.

Group A male and B female (the control and the group that took *Moringa oleifera before* 3-Nitropropionic acid) covered the highest distance when compared to the group D that took *Moringa oleifera after* 3-Ntropropionic acid covered the lowest distance.

MEAN+SEM	CONTROL	MO+N	N	N+MO
MALE	67.04±2.09	62.44±2.16	51.93±2.02	55.20±3.03
FEMALE	60.73±0.09	64.53±1.36	54.48±0.91	40.08±6.26



Distance TRIALS FEMALE

GROUP DETAILS	MEAN DIFF	SE DIFF	SIGNIFICANT	P-VALUE
Control—MO+N	-3.80	1.42	YES	0.05
Control—N	6.25	0.94	NO	0.07
Control—N+MO	20.65	6.18	YES	0.02
MO+N—N	10.05	4.39	YES	0.00
MO+N—N+MO	24.45	7.08	YES	0.02
N—N+MO	14.40	6.58	NO	0.09

DISTANCE TRIALS MALE

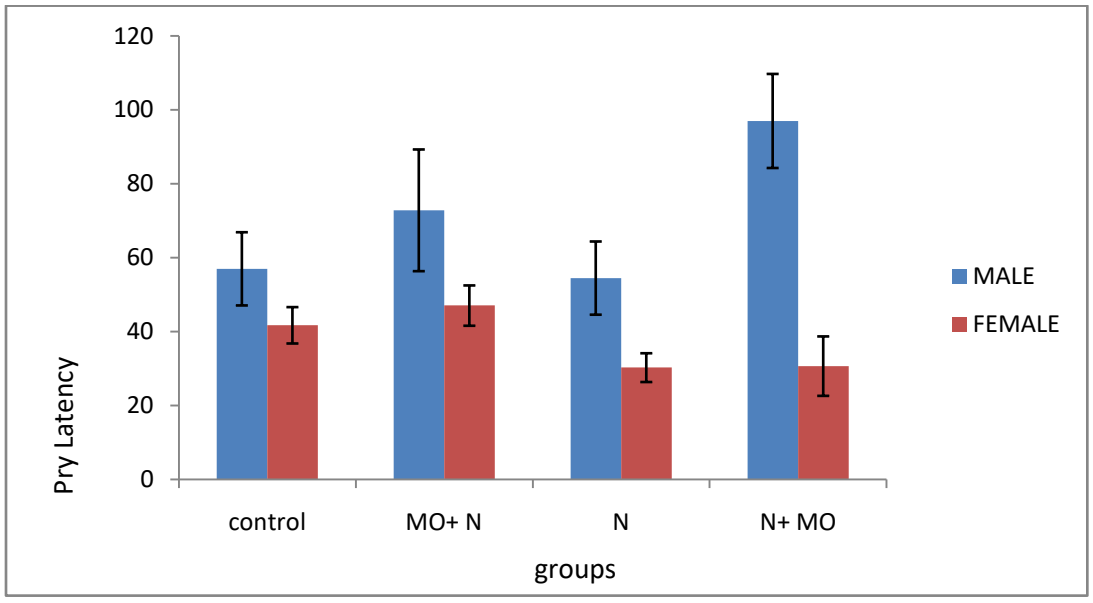
GROUP DETAILS	MEAN DIFF	SE DIFF	SIGNIFICANT	P-VALUE
Control—MO+N	4.60	3.28	NO	0.23
Control—N	18.10	2.59	NO	0.24
Control—N+MO	11.84	4.59	YES	0.00
MO+N—N	10.50	3.17	YES	0.00
MO+N—N+MO	7.24	8.44	NO	0.12
N—N+MO	-3.26	8.09	NO	0.14

Primary latency of male and female mice after administration

The result from each group are varied, the group D male and C female that took *Moringa oleifera* before and after 3-Nitropropionic acid have increased in primary latency when compare with other groups and group C male and female that took only the drug(3-Nitropropionic acic) have the lowest primary latency relative to the control group, these differences were not statistically significant.

PRY LATENCY TRIALS DETAILS

MEAN+SEM	CONTROL	MO+N	N	N+MO
MALE	57.00±9.90	72.83±16.46	54.50±9.90	97.00±12.72
FEMALE	41.73±4.92	47.06±5.45	30.28±3.89	30.68±8.04



PRY LATENCY TRIALS MALE

GROUP DETAILS	MEAN DIFF	SE DIFF	SIGNIFICANT	P-VALUE
Control—MO+N	-15.83	16.48	NO	0.38
Control—N	2.50	16.36	NO	0.88
Control—N+MO	-40.00	21.00	NO	0.11
MO+N—N	18.33	13.68	NO	0.23
MO+N—N+MO	-24.16	24.32	NO	0.36
N—N+MO	-42.50	19.35	NO	0.08

PRY LATENCY TRIALS FEMALE

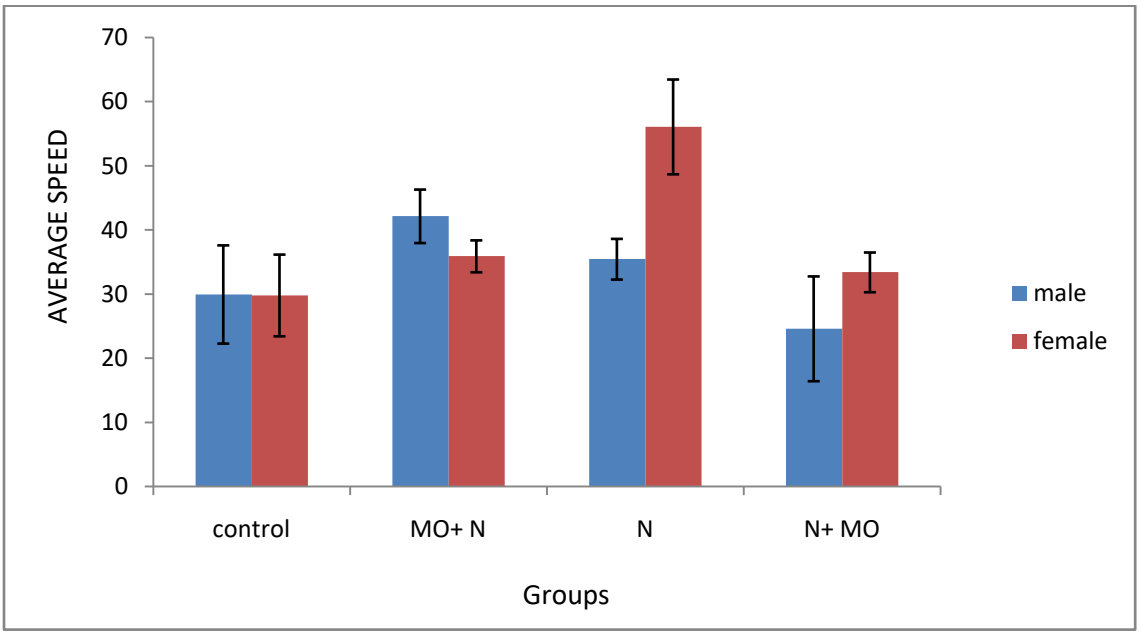
GROUP DETAILS	MEAN DIFF	SE DIFF	SIGNIFICANT	P-VALUE
Control—MO+N	-5.33	4.00	NO	0.25
Control—N	11.45	5.90	NO	0.12
Control—N+MO	11.05	7.61	NO	0.22
MO+N—N	16.78	6.57	NO	0.06
MO+N—N+MO	16.38	4.24	YES	0.01
N—N+MO	0.40	9.25	NO	0.96

AVERAGE SPEED OF MALE AND FEMALE MICE

The female mice had increase in average speed throughout the groups when compare with the male groups. These differences were not statistically significant (P≥0.05).

AVERAGE SPEED MALE AND FEMALE LATENCY

Groups MEAN+ SEM	CONTROL	MO+N	N	N+MO
MALE	29.96±7.66	42.15±4.17	35.46±3.17	24.61±8.61
FEMALE	29.81±6.37	35.91±2.49	56.09±7.39	33.41±3.10



AVERAGE SPEED LATENCY TRIALS FEMALE

GROUP DETAILS	MEAN DIFF	SE DIFF	SIGNIFICANT	P-VALUE
Control—MO+N	-6.09	6.67	NO	0.41
Control—N	-26.27	10.17	NO	0.06
Control—N+MO	-3.59	5.71	NO	0.56
MO+N—N	2.49	2.56	NO	0.38
MO+N—N+MO	22.67	7.78	YES	0.04

AVERAGE SPEED LATENCY TRIAL MALE

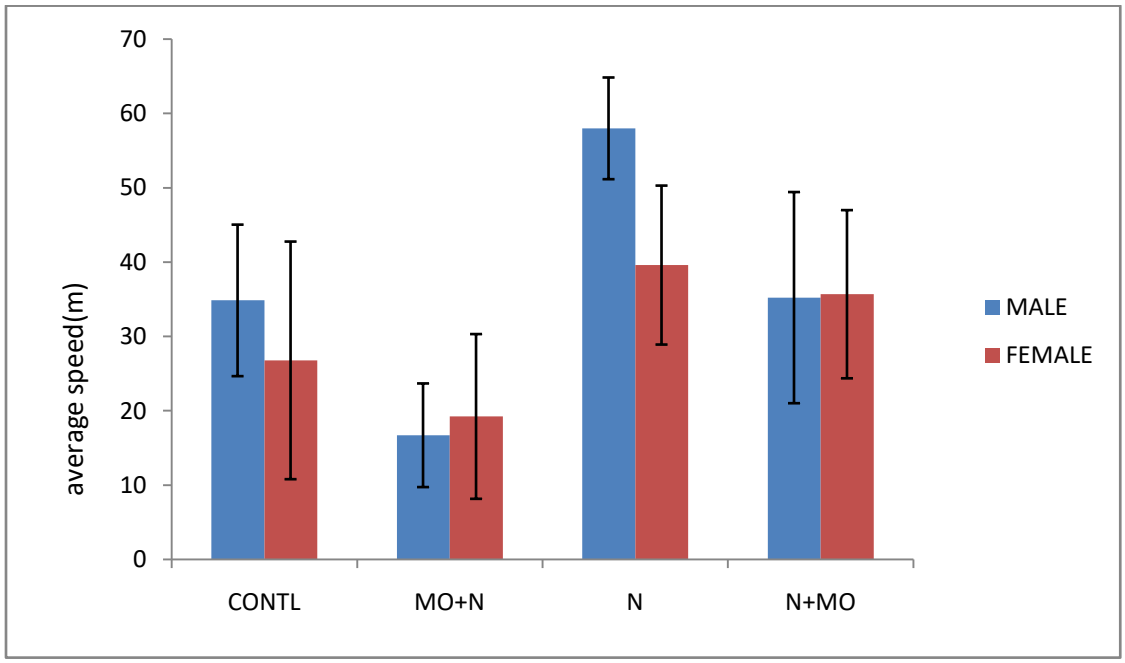
GROUP DETAILS	MEAN DIFF	SE DIFF	SIGNIFICANT	P-VALUE
Control—MO+N	-12.18	5.44	NO	0.08
Control—N	-5.49	10.31	NO	0.62
Control—N+MO	5.35	4.06	NO	0.25
MO+N—N	6.69	6.57	NO	0.36
MO+N—N+MO	17.53	7.74	NO	0.08
N—N+MO	10.84	10.07	NO	0.34

AVERAGE SPEED IN MICE (PROBE)

The graph shown the average weight for the final probe of all the groups at the end of the experiment the average speed of the animals in each groups were varied, the female group that took only the drug(3-Ntropropionic Acid) had the highest average speed compared to the other groups and they are not statistically significant.

THE TABLE BELOW SHOWN THE SPATIAL MEMORY (AVERAGE SPEED PROBE) IN EXPERIMENTAL GROUPS

GROUPS MEAN±SEM	CONTROL	MO+N	N	N+MO
MALE	26.79±15.98	19.25±11.08	39.61±10.69	35.69±14.20
FEMALE	34.86±18.62	16.72±6.97	58.00±6.84	35.23±14.2



AVERAGE SPEED DETAILS

TEST DETAILS (F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	18.13	16.76	NO	0.34
Control--- N	-23.14	15.28	NO	0.20
Control—N +Mo	-37.00	10.57	NO	0.97
Mo + N---N	-41.27	6.13	YES	0.03
Mo + N---N+ Mo	-18.50	19.95	NO	0.40
N --- N +MO	22.77	15.45	NO	0.21

TEST DETAILS(M)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	7.53	20.23	NO	0.72
Control--- N	-12.83	16.36	NO	0.47
Control—N +Mo	-8.90	19.86	NO	0.67
Mo + N---N	-20.35	15.23	NO	0.25
Mo + N---N+ Mo	-16.44	15.60	NO	0.35
N --- N +MO	3.91	4.36	NO	0.42

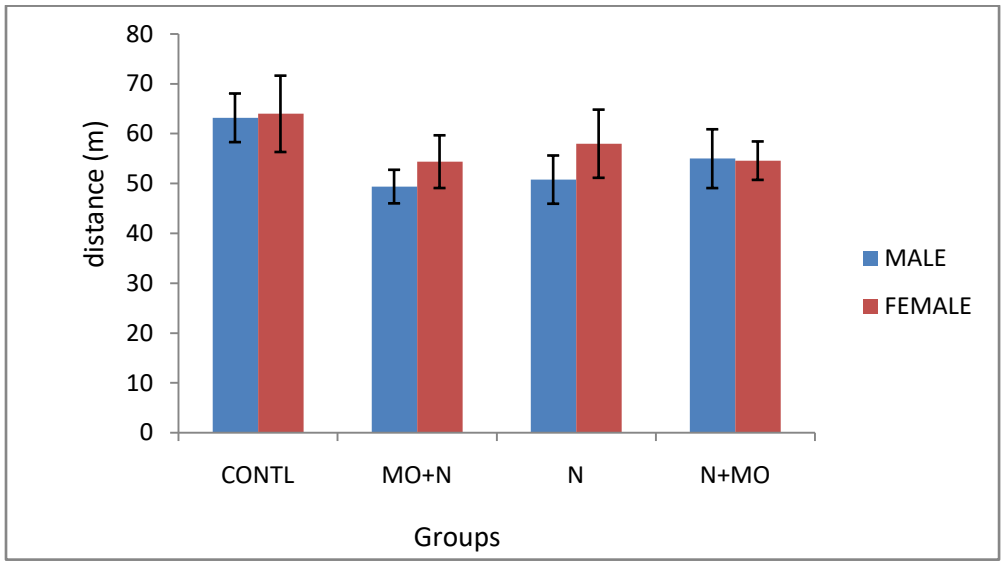
DISTANCE COVERED IN MALE MICE (PROBE)

Locomotors activities of all the groups were taken after the administration.

At the end of the distance speed the control group (male and female) cover the high distance but the control group (female)cover the highest while the other groups are decreasing and are not statistically significant .

THE GRAPH BELOW SHOWN THE MEAN AND SEM OF SPATIAL MEMORY DISTANCE (PROBE) OF MALE AND FEMALE AFTER EXPERIMENT.

GROUPS MEAN±SEM	CONTROL	MO+N	N	N+MO
MALE	63.20±4.88	49.40±3.37	50.80±4.84	55.00±5.90
FEMALE	64.00±7.66	54.40±5.29	58.00±6.84	54.60±3.86



DISTANCE DETAILS

TEST DETAILS (F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	9.60	12.91	NO	0.49
Control--- N	6.00	8.31	NO	0.51
Control—N +Mo	9.40	7.85	NO	0.29
Mo + N---N	-3.60	9.74	NO	0.73
Mo + N---N+ Mo	-0.20	7.08	NO	0.97
N --- N +MO	3.40	5.86	NO	0.59

TEST DETAILS(M)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	13.80	6.03	NO	0.08
Control--- N	12.40	7.98	NO	0.19
Control—N +Mo	8.20	10.29	NO	0.47
Mo + N---N	-1.40	6.60	NO	0.84
Mo + N---N+ Mo	-5.60	5.89	NO	0.39
N --- N +MO	-4.20	8.88	NO	0.57

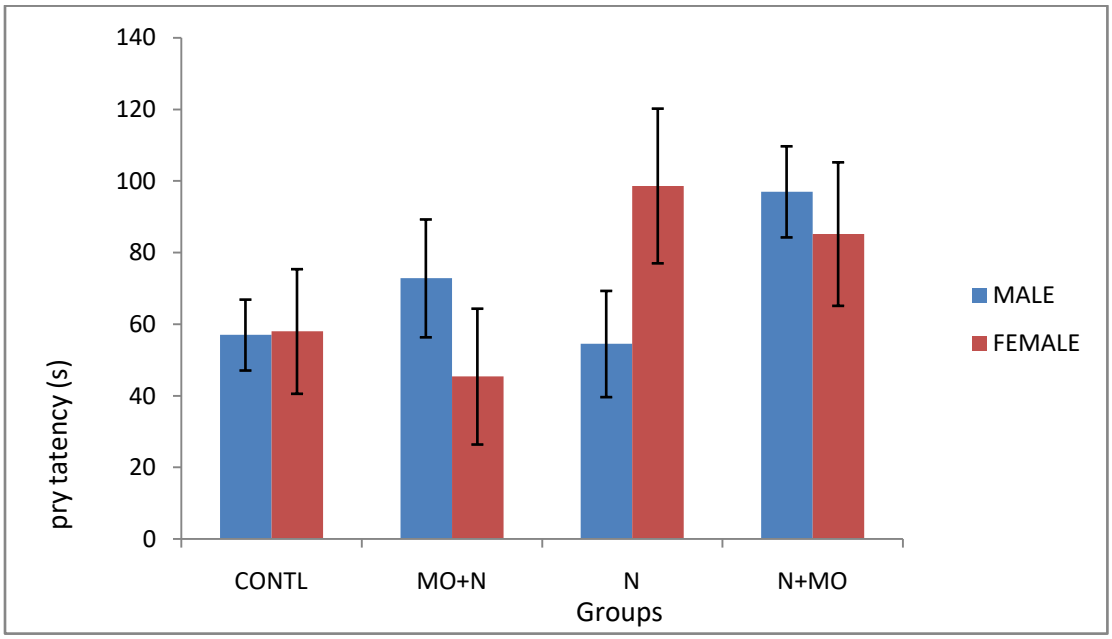
PRIMARY LATENCY (MALE AND FEMALE) (PROBE)

As a memory attribute of all the groups were taken after the administration.

At the end of the experiment the PL of the group that took *moringa oleifera* before the drug in the male group and the control group in the female group (male and female) cover the high distance while the other groups are decreased when compare with the control group.The differences between the control and most of the groups were not statistically significant.

THE GRAPH BELOW SHOWN THE MEAN AND SEM OF SPATIAL MEMORY PL (PROBE) OF MALE AND FEMALE AFTER EXPERIMENT

GROUP MEAN±SEM	CONTROL	MO+N	N	N+MO
MALE	57.00±9.90	72.83±16.46	54.50±14.83	39.20±25.75
FEMALE	58.00±17.49	45.40±18.67	38.64±2.40	37.20±20.75



PRY LATENCY (PROBE) DETAILS

TEST DETAILS(M)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	-15.83	16.38	NO	0.38
Control--- N	2.50	16.36	NO	0.88
Control—N +Mo	-40.00	21.00	NO	0.11
Mo + N---N	18.33	13.68	NO	0.23
Mo + N---N+ Mo	-24.16	24.32	NO	0.36
N --- N +MO	-42.50	19.35	NO	0.08

TEST DETAILS(F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	12.60	31.75	NO	0.71
Control--- N	19.36	27.74	NO	0.52
Control—N +Mo	-40.40	27.74	NO	0.21
Mo + N---N	6.76	32.09	NO	0.84
Mo + N---N+ Mo	-53.00	23.72	NO	0.08
N --- N +MO	-59.76	25.10	NO	0.07

OPEN FIELD WALLING FEMALE AND MALE MICE

The group that took only the drug had highest level when compared with other groups in th female groups. while the control group in male had the highest when compared with the other groups while the group that took *moringa oleifera* before the drug and the group that took *moringa oleifera* after the drug in the male group also had the lowest

THE TABLE BELOW SHOWING MEAN AND SEM OF THE **WALLING** IN MALE AND FEMALE EXPERIMENTL GROUPS

GROUPS MEAN±SEM	CONTROL(WALLING)	MO+N	N	N+MO
MALE	16.66±1.54	12.60±1.60	14.31±1.04	8.31±0.53
FEMALE	29.00±1.14	6.64±1.11	10.00±0.83	10.60±2.31

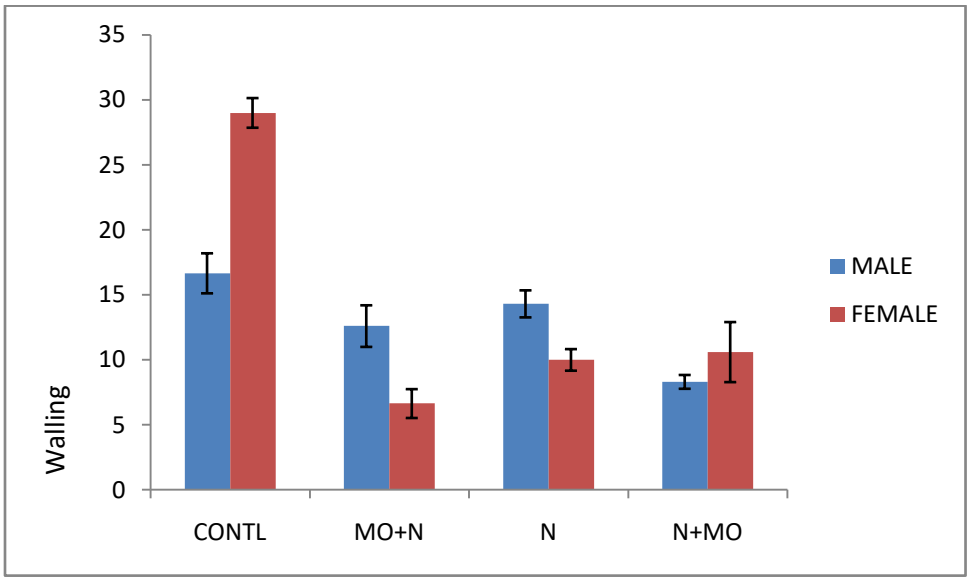


Fig4.6 above showing the grooming of the male and female experimental animals

WALLING DETAILS

TEST DETAILS(F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	22.36	1.85	YES	0.00
Control--- N	19.00	1.26	YES	0.00
Control—N +Mo	18.40	2.69	YES	0.02
Mo + N---N	-3.30	1.40	NO	0.07
Mo + N---N+ Mo	-3.96	2.43	NO	0.17
N --- N +MO	-0.60	2.65	NO	0.83

TEST DETAILS(M)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	4.06	2.08	NO	0.10
Control--- N	2.35	2.10	NO	0.31
Control—N +Mo	8.35	1.57	YES	0.03
Mo + N---N	-1.71	1.90	NO	0.41
Mo + N---N+ Mo	4.28	1.76	NO	0.06
N --- N +MO	6.00	1.57	YES	0.01

OPEN FIELD GROOMING IN MALE AND FEMALE MICE

The control female had the highest when compared with other groups. The female group the that took only the drug had the lowest level

THE TABLE BELOW SHOWING MEAN AND SEM OF THE **GROOMING** IN MALE AND FEMALE EXPERIMENTAL GROUPS

GROUPS MEAN±SEM	CONTROL	MO+N	N	N+MO
MALE	8.00±1.06	4.60±1.45	9.31±0.39	5.00±0.68
FEMALE	9.64±1.49	5.32±1.74	4.40±1.02	7.80±1.85

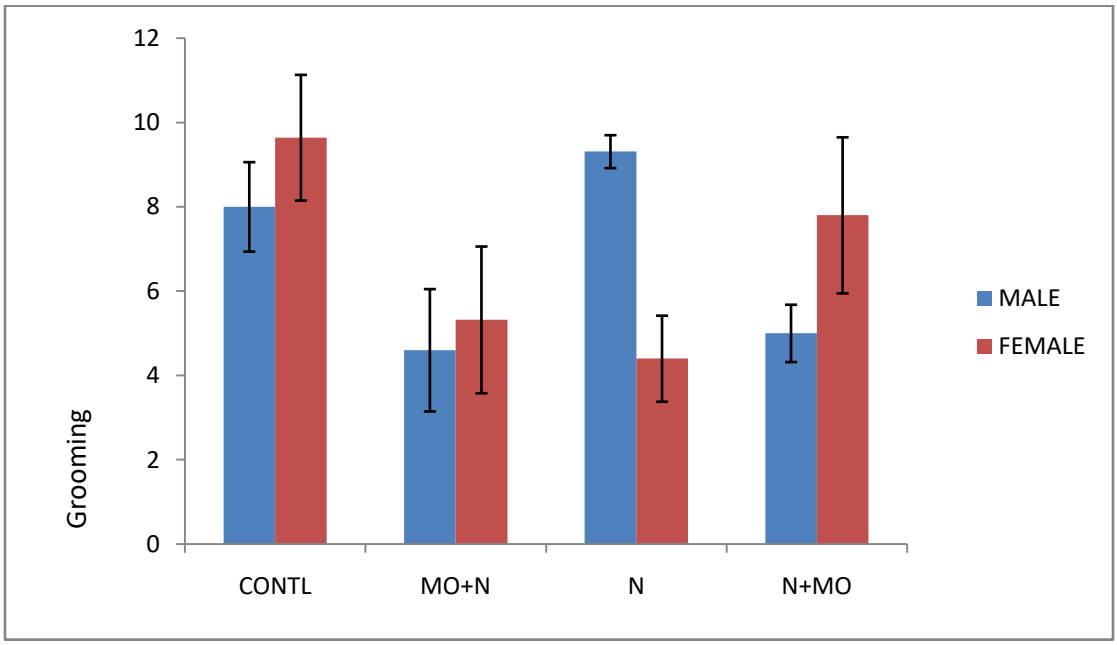


Fig4.6 above showing the grooming of the male and female experimental animals

GROOMING DETAILS

TEST DETAILS(F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	-15.83	16.38	NO	0.38
Control--- N	12.50	16.36	NO	0.88
Control—N +Mo	-40.00	21.00	NO	0.11
Mo + N---N	18.33	13.68	NO	0.23
Mo + N---N+ Mo	24.16	24.32	NO	0.36
N --- N +MO	-42.50	19.35	NO	0.08

TEST DETAILS(M)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	3.40	1.30	YES	0.04
Control--- N	-1.31	1.16	NO	0.31
Control—N +Mo	3.00	0.93	YES	0.02
Mo + N---N	-4.71	1.41	YES	0.21
Mo + N---N+ Mo	-0.40	1.64	NO	0.81
N --- N +MO	4.31	1.04	YES	0.09

OPEN FIELD (MALE AND FEMALE REARING) MICE

The each group are varied among the animal. The control male group and the that took *moringa oleifera* after 3-Nitropropionic acid had the higher but when compare the control group had the highest while there is reductionin other groups.

THE TABLE BELOW SHOWING MEAN AND SEM OF REARING IN MALE AND FEMALE EXPERIMENTL GROUPS

GROUPS MEAN±SEM	CONTROL	MO+N	N	N+MO
MALE	13.20±3.15	5.60±2.87	4.64±0.79	9.64±1.11
FEMALE	4.92±1.22	4.64±0.79	8.6±3.29	8.840±1.77

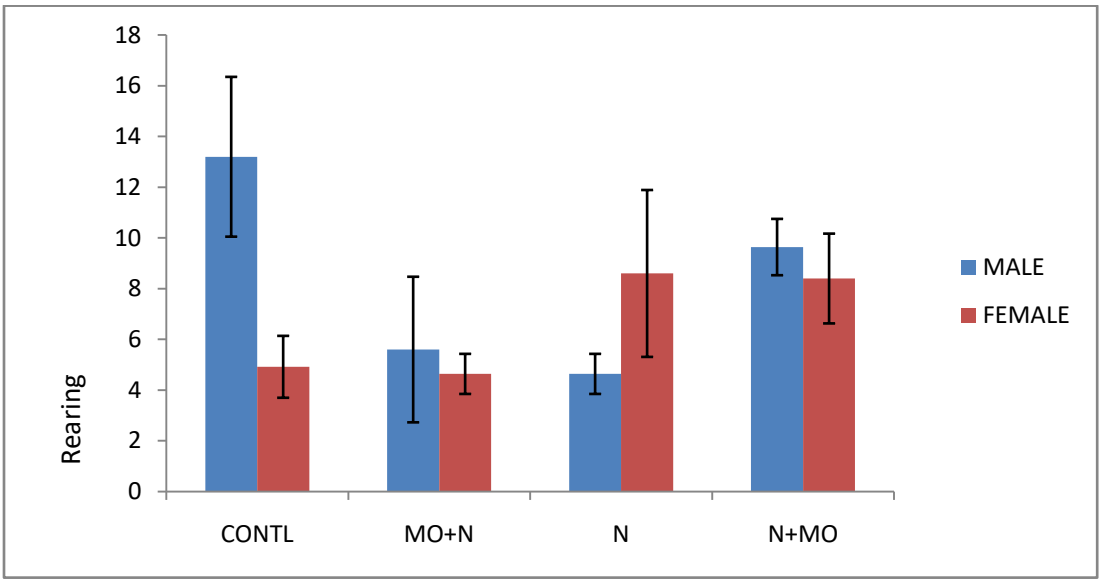


Fig4.6 above showing the rearing of the male and female experimental animal

REARING DETAILS

TEST DETAILS(F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	0.28	1.94	NO	0.89
Control--- N	-3.68	3.42	NO	0.34
Control—N +Mo	-3.48	2.44	NO	0.22
Mo + N---N	-3.96	3.48	NO	0.31
Mo + N---N+ Mo	-3.76	1.90	NO	0.12
N --- N +MO	0.20	2.31	NO	0.93

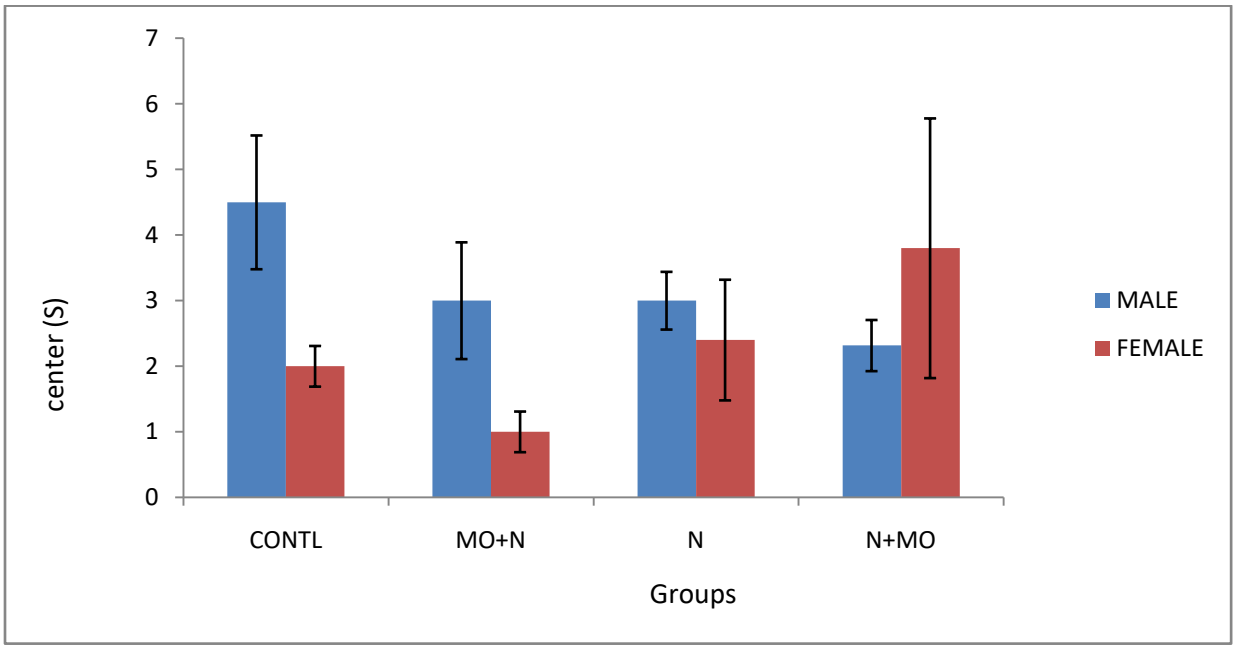
TEST DETAILS(M)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	7.60	1.66	YES	0.01
Control--- N	8.56	3.88	NO	0.09
Control—N +Mo	3.56	2.77	NO	0.26
Mo + N---N	0.96	3.62	NO	0.80
Mo + N---N+ Mo	-4.04	2.12	NO	0.13
N --- N +MO	-5.00	1.67	YES	0.04

OPEN FIELD (TIME SPENT AT THE CENTER SQUARE BY MALE AND FEMALE MICE)

The center squared in each group is varied among the animal. The control male group and the that took *moringa oleifera* after 3-Nitropropionic acid had the higher the group that took *moringa oleifera* before 3- Nitropropionic acid in female group had the lowest center square when compared with other groups.

THE TABLE BELOW SHOWING MEAN AND SEM OF CENTER SQUARE IN MALE AND FEMALE EXPERIMENTL GROUPS

GROUPS MEAN±SEM	CONTROL	MO+N	N	N+MO
MALE	4.50±1.02	3.00±0.89	3.00±0.44	2.31±0.39
FEMALE	2.00±0.31	1.00±0.316	2.40±0.92	3.80±1.98



CENTER SQUARE DETAILS

TEST DETAILS(F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	1.00	0.31	YES	0.03
Control--- N	-0.40	1.02	NO	0.71
Control—N +Mo	-1.80	2.05	NO	0.43
Mo + N---N	-1.40	1.02	NO	0.24
Mo + N---N+ Mo	-2.80	2.03	NO	0.24
N --- N +MO	-1.40	1.91	NO	0.50

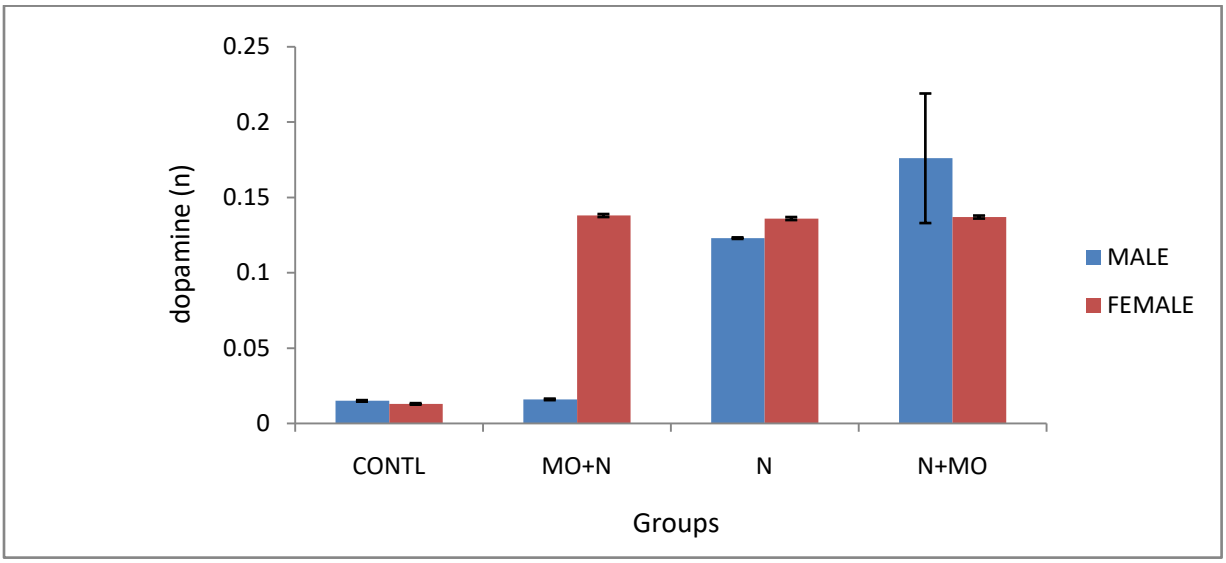
TEST DETAILS(M)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	1.50	0.71	NO	0.09
Control--- N	1.50	1.40	NO	0.33
Control—N +Mo	2.18	0.88	YES	0.05
Mo + N---N	OE-7	1.23	NO	1.00
Mo + N---N+ Mo	0.68	0.57	NO	0.28
N --- N +MO	0.68	0.78	YES	0.42

DOPAMINE LEVEL IN MALE AND FEMALE MICE

Dopamine was generally observed to be higher in treated and untreated groups relative to the Control Group in both sexes. Group D male had the highest dopamine level (that took *Moringa oleifera* before 3-Nitropropionic Acid) control group of male and female had the also lower in Dopamine level. Group B Male (that is the group that took moringa before 3-Nitropropionic acid were statistically dfference)

THE TABLE BELOW SHOWING MEAN AND SEM OF THE DOPAMINE LEVEL IN FEMALE EXPERIMENTL GROUPS

GROUPS MEAN±SEM	CONTROL	MO+N	N	N+MO
MALE	0.015±0.0005	0.016±0.0005	0.123±0.005	0.176±0.043
FEMALE	0.013±0.0005	0.138±0.0010	0.136±0.0010	0.137±0.0010



Shows Dopamine analysis in homogenate of experimental groups in male and female animals

Dopamine Details

TEST DETAILS (M	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N			NO	0.22
Control--- N	0.00	0.00	NO	0.0
Control—N +Mo	-0.16	0.00	NO	0.06
Mo + N---N	-0.10	0.00	YES	0.00
Mo + N---N+ Mo	-0.16	0.04	NO	0.06
N --- N +MO	-0.05	0.04	YES	0.34

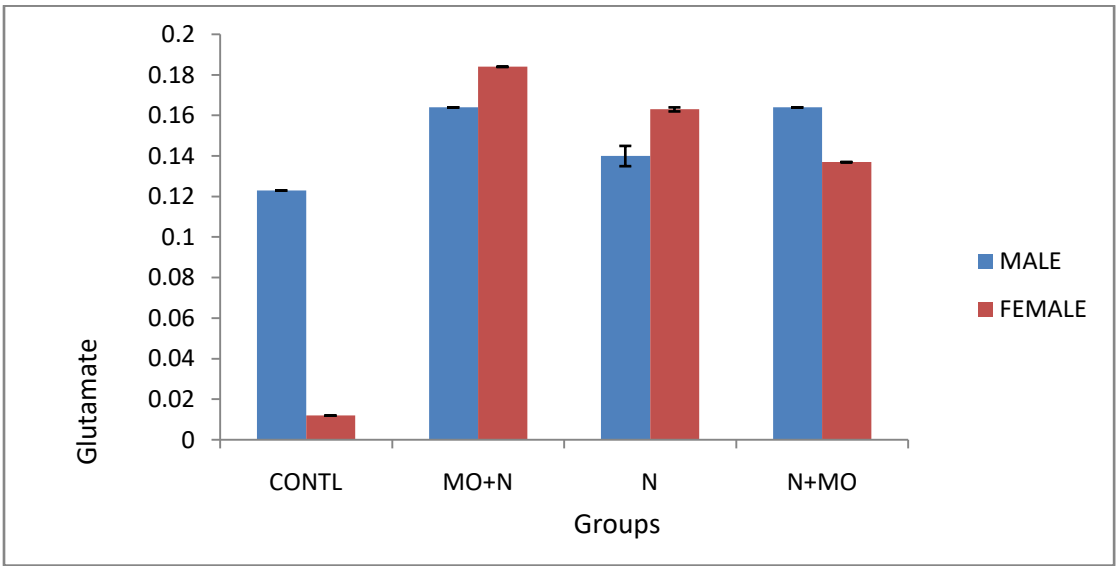
TEST DETAILS (F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	9.60	12.91	NO	0.49
Control--- N	6.00	8.31	NO	0.51
Control—N +Mo	9.40	7.85	NO	0.29
Mo + N---N	-3.60	9.70	NO	0.73
Mo + N---N+ Mo	-0.20	7.08	NO	0.97
N --- N +MO	3.40	5.86	NO	0.59

GLUTAMATE LEVEL IN FEMALE AND MALE MICE

Glutamate was generally observed, the control group female had the lowest level relative to the other Group and the differences were statistically significant. The group B female that took the *moringa oleifera* for 7 days and 3-Nitropropionic Acid for another 7days had the highest,glutamate level while most of the female are greater than the male in each groups

THE TABLE BELOW SHOWING MEAN AND SEM OF THE GLUTAMATE LEVEL IN MALE AND FEMALE EXPERIMENTL GROUPS

GROUPS MEAN±SEM	CONTROL	MO+N	N	N+MO
MALE	0.1230±0.0000	0.1640±0.0005	0.1400±0.0057	0.1640±0-0005
FEMALE	0.0120±0.0005	0.1835±0.0050	0.1632±0.0015	0.1370±0.0003



shows Glutamate analysis in homogenate of experimental group of male and female animals

Dopamine Details

TEST DETAILS (F)	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	-0.17	0.00	YES	0.00
Control--- N	-0.15	0.00	NO	0.00
Control—N +Mo	-0.12	0.00	YES	0.00
Mo + N---N	0.20	0.00	YES	0.00
Mo + N---N+ Mo	0.46	0.00	YES	0.00
N --- N +MO	0.25	0.00	YES	0.00

TEST DETAILS (M	MEAN DIFF.	SE DIFF.	SIGNIFICANT	P.VALUE
Control---Mo+ N	-0.15	0.00	Yes	0.00
Control--- N	-0.12	0.00	Yes	0.02
Control—N +Mo	-0.13	0.01	No	0.00
Mo + N---N	0.02	0.00	Yes	0.04
Mo + N---N+ Mo	0.02	0.01	NO	0.14
N --- N +MO	0.00	0.00	NO	0.82

IV. DISCUSSION

This study indicate the effect of both prophylactic and curative action of aqueous extract of *Moringa Olefera* on 3-Nitriopriopinic acid induced huntington's diseases. Huntington's disease (HD) is a neurodegenerative genetic disorder that affects muscle coordination and leads to mental decline and behavioral symptoms (16) Symptoms of the disease can vary between individuals and affected members of the same family, but usually progress predictably. The earliest symptoms are often subtle problems with mood or cognition. A general lack of coordination and an unsteady gait often follows. As the disease advances, uncoordinated, jerky body movements become more apparent, along with a decline in mental abilities and behavioral symptoms. (16) Physical abilities gradually worsen until coordinated movement becomes difficult.

As HD progresses, there is strong impairment in motor functions that alter muscular movements and reduced locomotor activity (23). As per the reports intra-peritoneal administration of 3-NP cause muscular atrophy which leads to decrease in locomotion counts. Reduced number of locomotion also indicates central nervous system (CNS) depression which is one of the symptoms of HD. In the present study, administration of 3-NP produced significant decreased in locomotions. These are in agreement of the previous reports.

As the disease progresses swallowing become more difficult thereby decreased intake of calories results in body weight loss as observed in this study. This is in agreement with the other studies that administration of 3-NP produced significant decreased in weight (24)

Neurobehavioral study strengthened the fact that HD reduces learning, memory and cognition; this was evidence in Barnes maze test for learning and memory. Treated animals were found to have higher primary latency compared to the control group although group given MO and 3-niropropionic acid showed slightly different in primary latency when compared with group that took only 3-nitropropionic acid. One can therefore say that aqueous extract of MO reduces the effect of HD on memory and learning.

Locomotor activities test was also done to seen the effect of 3-nitropriopionic acid induced Huntington Disease on locomotion. We found out in open field test as well as Barnes maze test conducted that there was greater decreased in distance covered by the treated group relative to the control group although the distance covered was found to be higher in the group treated with Moringa as well as 3-nitropropionic acid relative to the group that received only 3-nitropropionic acid.

The most recent evidence points to decreases in Dopamine (DA) and glutamate neurotransmission as the HD phenotype develops. However, there is some evidence for increased DA and glutamate functions that could be responsible for some of the early HD phenotype. Significant evidence indicates that glutamate and dopamine neurotransmission is affected in HD, compromising the fine balance in which DA modulates glutamate-induced excitation in the basal ganglia and cortex. Restoring the balance between glutamate and dopamine could be helpful to treat HD symptoms (R). This study shows that there is an increase level of both Dopamine and glutamate on the 3-Nitriopropionic acid treated rats which connote the manifestation of HD. These could not be reversed by both the prophylactic and curative treatment of *Moringa Olefera.*

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