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Evaluation of Neuroprotective Activity of Moringa Concanensis Leaves Extract in Rats

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ABSTRACT: The present study was designed to evaluate the Neuroprotective properties ofethanolic plant extract of Moringa concanensis (M c).The Neuroprotective activity of ethanolic leaves extract of Moringa concanensis was evaluated for protection against Alcl3 (50 mg/kg p.o for 7 days) and 3-NP (10 mg/kg i.p for 7 days) induced Neurotoxicity in albino rats. The behavioural activities along with the body weight, biochemicals (Lipid peroxidation, Superoxide dismutase, Glutathion, and Total protein) and histopathological studies were carried out on Brain of experimental animals was considered to determine the Neuroprotective property.

I. INTRODUCTION

Nervous system

The nervous system is the most intricate bodily organ in humans and other vertebrates. The nervous system, which is the body's primary coordinating and communication network, is always alive with electricity. It is so large and intricate that it has been calculated that if all the individual nerves from one body were linked end to end, they could circle the globe 2.5 times.[1] The two main divisions of the nervous system are the peripheral nervous system (PNS) and the central nervous system (CNS).[2]

The Central Nervous System is made up of the Brain and Spinal Cord. The Peripheral Nervous System is made up of nerves and sensory organs. The peripheral nervous system (PNS) and central nervous system (CNS) work in concert to transmit, process, and coordinate body processes. The control center is comprised of the brain and spinal cord (the CNS). They gather information and input from the body's sensory organs and nerves, process it, and then send commands back out. The PNS's nervous systems are responsible for sending and receiving messages.[1]

FUNCTIONS OF NERVOUS SYSTEM

1. Gathers data from the body's inside and exterior - Sensory Function
2. Sends data to the brain and spinal cord's processing regions.
3. Processes data from the spine and brain - Integration Function
4. Communicates with the muscles, glands, and organs to enable them to react appropriately - Motor Function

It directs and coordinates all bodily processes necessary for maintaining homeostasis, or the delicate balance of the body, including those of all other bodily systems.[2]

The neuron is the basic structural and functional component of the brain and central nervous system. Electrical and chemical signals are sent by neurons, which are specialized cells. Only glial cells, which provide structure and support for neurons, are included in the list of cells that make up the brain. Together, the 86 billion neurons that make up the nervous system link to the rest of the body. From hunger and pain to consciousness and thought, they are in command of everything. There are three main types of neuron: sensory neurons, motor neurons, and interneurons.[3]

Neuron

At one end of the cell body and over the bulk of its periphery, dendrites are a variety of small, branching protrusions. The axon hillock is the place where the axon, a long, thin, tube-like protrusion, emerges from the opposite end of the cell body. The axon is encased in myelin.[4]

Neurotoxicity

Neurotoxicity is the capacity to have adverse effects on the central nervous system, peripheral nerves, or sensory organs. A chemical is considered neurotoxic if it can result in a repeated pattern of neurological malfunction or a change in the chemistry or structure of the nervous system.[5]

Neurotoxicity is the term used to describe the alteration of the nervous system's normal function as a result of exposure to dangerous substances (neurotoxicants). As a result, neurons—vital cells that transmit and process information in the brain and other regions of the nervous system—

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might eventually sustain harm or even perish. Neurotoxicity can be caused by exposure to chemicals used in chemotherapy, radiation therapy, drug therapies, organ transplants, certain foods and food additives, pesticides, industrial and/or cleaning solvents, cosmetics, and some naturally occurring substances.[6]

Protein misfolding and aggregation in chronic neurodegenerative diseases:

• The term "misfolding" refers to the adoption of aberrant conformations by some regularly produced proteins, which cause them to frequently form massive, soluble aggregates.

Misfolded proteins frequently have hydrophobic surface residues that encourage aggregation and association with membranes. Initially, these aggregates are large, soluble oligomers that accumulate intracellularly or extracellularly as microscopic deposits, which are stable and proteolysis-resistant. Although the mechanisms are not clear, such aggregates, or the misfolded protein precursors, lead to neuronal death.[7]

Effects of Neurotoxicity

Some of the effects of neurotoxicity may appear immediately, while others can take months or years to manifest.

The effects of neurotoxicity depends on various different factors such as the characteristics of the neurotoxin, the dose a person has been exposed to, ability to metabolize and excrete the toxin, the ability of affected mechanism and structures to recover and how vulnerable a cellular target is.[8]

Some of the symptoms of neurotoxicity include:

- Loss of circulation
- Imbalance
- Flu-like symptoms
- Brain damage
- Memory loss
- Anxiety
- Depression[8]

A progressive loss of neurons' structural and functional integrity known as neurodegeneration results in a variety of clinical and pathological manifestations before the functional architecture deteriorates. Numerous neurodegenerative disorders, which can be distinguished based on their various pathological mechanistic pathways, are frequently caused by this progressive neuronal cell death, including Parkinson's disease (PD), Huntington's disease (HD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), brain trauma (BT), prion disease (PrD), progressive supranuclear palsy (PSP), and spinocerebellar ataxias (SCA). It comprises related neuropathology, anatomical susceptibility due to the disease, and the aggregation of some key selective proteins under pathological conditions.[9]

The degeneration of CNS is characterized through chronic revolutionary loss of the shape ,features of neuronal materials, consequent in persistent and mental impairments. Neurodegenerative diseases are characterised by the lack of neurons and modern dysfunction main to the first rate involvement of sensible systems defining scientific displays. The pathological additives involve the permeability of the blood brain barrier, the harm of myelin sheath, axon injury, the glial scar formation and the incidence of inflammatory cells, usually lymphocytes are infiltrated into the CNS. The lack of myelin is manifested in scientific symptoms together with neuropathic pain, paralysis, muscle spasms and opticneuritis.[10]

Mechanism of neurodegeneration

Common pathogenic mechanisms underlying many NDDs include:

1) Abnormal protein dynamics with misfolding, defective degradation, proteasomal dysfunction and aggregation; often with actions and mutations of molecular chaperones.
2) Oxidative stress (OS) and formation of free radicals/reactive oxygen species (ROS).
3) Impaired bioenergetics, mitochondrial dysfunctions and DNA damage.
4) Fragmentation of neuronal Golgi apparatus
5) Disruption of cellular/axonal transport.[11]

Objectives

1. Collection and Authentication of Moringa concanensis (M c) plant.
2. Preparation of ethanolic extract of M c by soxhlet apparatus.
3. Preliminary phytochemical investigation from obtained extract.
4. Evaluation of Neuroprotective activity of M c extracts on Aluminium chloride(Alcl3) and 3-Nitropropionic acid (3-NP) induced neurotoxicity in rats.

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5. Evaluation of following parameters in Alcl3 and 3-NP induced Neurotoxicity.

I. Measurements of body weight change.

Behavioral tests:

The following observations will be recorded.

1. Elevated plus maze test.
2. Locomotor activity.
3. Rota-rod performance assessment for motor co-ordination.
4. String test for Grip strength.

II. Brain tissue biochemical estimation: The following parameters will be estimated.

1) Glutathione (GSH).
2) Superoxide dismutase (SOD).
3) Lipid peroxidation (LPO).
4) Protein estimation.

Brain tissue histopathological changes will be observed

Plant profile:

Moringa concanensis Nimmo is a medicinal plant belonging to the family Moringaceae (fig1), which is called as Kattumurungai or Peyimurungai in Tamil. It is present in large amount in the district of Perambalur, TamilNadu.[12]

Figure 1: Picture of Moringa concanensis plant[13]

The plant Kattumurungai is entirely different from the Murungai (Moringa oleifera). Leaves and flowers are larger in size than M. oleifera. The appearance of bark shows distinct feature in both the species of Moringa. Bark is very smooth and is very hard in both the plants respectively. The horse radish odour of M. concanensis is more intense than M. oleifera. M. concanensis has a strong central trunk that is covered with an extremely distinctive layer of very furrowed bark that can be more than 15 cm thick.[12]

Taxonomical classification:

1. Species: Moringa concanensis Nimmo
2. Family: Moringaceae
3. Kingdom: Plantae
4. Phylum: Tracheophyta
5. Class: Magnoliopsida
6. Order: Capparales
7. Genus: Moringa
8. Common name: Konkan moringa, Moringa.[14]

Pharmacognestic Description

Habit: A tree with rachis thickened at the base, gland present at articulation.[15]

Leaves: Bipinnate, leaflets 4-6 pairs and an odd one broadly elliptic, obtuse at both ends.

Flowers: Bracteate, bracts minute, calyx cup-shaped, white, oblong, reflexed.

Petals are yellow, veined with red, oblong.

Stamens: 5 fertile and 4-5 staminodes, filaments hairy at base.[16]

Inflorescence: Panicles.

Fruit: Capsules are straight, slightly constricted between the seeds. Seeds may be white or pale yellow, 3-angled, 3-winged, wings very thin.[17]

Medicinal and Therapeutic Uses:

- The juice of the fresh leaves is used to reduce cholesterol and body weight.
- It also helps to reduce blood pressure and prevent hypertension and cardiovascular diseases.
- Leaves of Moringa concanensis were collected, washed and cooked as a vegetable and taken internally twice in a week will produce cooling effect of eyes and prevent sore eyes.
- A paste prepared from the leaves and applied over the surface of body for 7 days helps as a remedy for jaundice.
- Used in the treatment of Skin tumor, Head ache, and eye diseases.
- The Decoction of the leaves is used to treat Diabetes.[18]

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Phytochemical Constituents:

The phytochemical analysis of Moringa concanensis are shown in Table below. The alkaloids are found to be present in all solvent extracts (Aqueous, Methanol, ethanol, chloroform and ethylacetate). The phytochemical investigation of all the extracts of M c revealed the presence of alkaloid, phenol, flavonoids, sterol, terpinoids, carbohydrates and protein; however alkaloid found to be more in ethanol, methanol and ethyl acetate extract than other solvents. Tannins found to be present in Aqueous, Ethanol extract and trace amount in other solvents. Among all the extracts of M. concanensis Nimmo leaves, chloroform, ethyl acetate and aqueous extracts have the lowest number of phytochemicals. The ethanolic extract of the leaves was found as a rich source of phytochemicals as compared to the other extracts. Thus the preliminary phytochemical analysis is more necessary for knowing the medicinal properties of plants.

The species of Moringa concanensis exhibited anti-microbial, anti-inflammatory and antioxidant properties due to presence of alkaloid, saponins, flavonoids, tannins and other phytochemicals.

Table1: Preliminary phytochemical analysis of Moringa concanensis.

Phytochemicals	Aqueousextract	Ethanol Extract	Methanolextract	Chloroformextract	Ethly acetate extract
Alkaloids Test	++	+++	+++	++	+++
Flavinoids Test	++	+++	++	-	-
Phenols test.	-	++	++	-	-
Steroids test.	+	++	++	+	-
Terpenoids test.	+	++	+	+	+
Carbohydrates test.	+	++	++	+	+
Proteins test.	++	++	++	+	+
Tannins test.	++	++	+	+	+

(+) = present in small concentration, (++) = present in medium concentration, (+++) = Present in high concentration, (--) = absent[19]

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Materials and method

Experimental animals: Albino wistar male rats of weight 180-220g were used in the experiment. Each group contains 6 animals.Animals were acclimatized for a week in experiment room. After acclimatization the animals were subjected to a gross observation to ensure that the selected rats were in good state of health. Rats were randomly selected for final allotment to the study.

Environmental condition: Air conditioned rooms with optimal air changes per hour, relative humidity, temperature and elimination cycle set to 12 hour light and 12 hour in dark. The animals were maintained under standard condition in an animal house approved by the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Mallige College of pharmacy, Bangalore.

Methodology

Collection and authentication of plant material

For the study, leaves of Moringa concanensis, collected from the nursery and local garden. The sample will be identified and authenticated by Dr P. E. Rajasekharan, Principal Scientist and Nodal officer GAC division of Plant Genetic Resources, Indian Institution of Horticultural Research, Hessarghatta Post, Bangalore-560089, and Karnataka, India.

Preperation of exract

The plant of Moringa concanensis will be collected and washed, chopped, dried at room temperature after which they will be made in to coarse powder. A weighed quantity (500 g) of the coarse powder was taken and extracted with ethanol (90%) in a Soxhlet apparatus. The extract was concentrated on a water bath at a temperature not exceeding 60ºC (yield 10% w/w). The ethanolic extract was suspended in distilled water.[20]

Fig.2 soxhlet apparatus

A. Preliminary phytochemical investigation:

The obtained plant extract will be subjected to phytochemical screening to detect the presence of various phytochemical viz. alkaloids, flavonoids, tannins, triterpenoids and saponins.

1. Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.
Mayer s test,Wagner s test are the two test used to detect alkaloids**.**

2. Test for Flavonoids

Lead acetate test and H_2SO_4 test was done to detect flavanoids

3. Test for Steroids

Two ml of acetic anhydride was added to five mg of the extracts, each with two ml of H_2SO_4. The colour was changed from violet to blue or green in some samples indicate that the presence of steroids.

4. Test for Terpenoids (Salkowski's test)

Five mg of the extract of the leaves, flowers and seeds was mixed with two ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. An appearance of reddish brown colour in the inner face was indicates that the presence of terpenoids.

5. Test for Anthroquinones

Borntrager s Test : About five mg of the extract was boiled with 10% Hcl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink colour indicates that the presence anthroquinones.

6. **Test for Phenols**

To 10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.

7. Test for Saponins

About 0.5mg of the extract was shaken with five ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows that the presence of saponins.

8. Test for Tannins

A small quantity of extract was mixed

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with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicates that the presence of tannins.

9. Test for Carbohydrates

0.5mg extracts were dissolved individually in five ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

10. Test for Proteins (Ninhydrin test)

About 0.5 mg of extract was taken and two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates that the presence of proteins, peptides or amino acids.[21]

9. Acute oral toxicity study:

Acute toxicity study was carried out according to Organization of Economic Co-Operation and Development (OECD) guidelines on Swiss albino mice, and the animals were kept overnight fasting providing water ad libitum, after which the extracts were administered orally 1600 mg/kgand observed for the mortality. Based on the results obtained from this study, the dose of further pharmacological studies was fixed to be 200 and 400mg/kg.[20]

Determination of Neuroprotective activity of the following animal model experiments are to be performed.

a) Aluminium chloride induced neurotoxicity.
b) 3-Nitropropionic(3-NP) acid induced neurotoxicity model.

Experimental Design

a) Aluminium chloride induced neurotoxicity model:

Total 30 rats were randomly divided into five groups of 6 rats each and treated for 7 days asfollows:

Table 2: Aluminium chloride induced neurotoxicity model:

Sl. No.	Group	Treatment	Duration of treatment
1.	Negative control	Normal Saline	Daily for 7days
2.	Positive control	$AlCl_3$ 50mg/kg (p.o.) + water (oral).	Daily for 7days
3.	Moringa concanensis (low dose : 200mg/kg)	$AlCl_3$ 50mg/kg (p.o.)+Test (Moringa concanensis)extract low dose (p.o.)	Daily for 7days
4.	Moringa concanensis (highdose: 400mg/kg)	$AlCl_3$ 50mg/kg (p.o) + Test (Moringa concanensis) extract high dose (p.o.) .	Daily for 7days
5	Standard (Piracetam)	$AlCl_3$ 50mg/kg(p.o) + Piracetam 200mg/kg (p.o)	Daily for 7days

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The Moringa concanensis extract was administered before 1hour administration of AlCl3 50mg/kg. During the drug treatment rats were observed for the behavioural changes for 50 minutes daily. On 8th day the rats were evaluated for General behavioural studies such as string test for Grip strength, Locomotor activity, Rota-rod performance assessment for motor coordination and Elevated plus maze test. On 9th day rats were sacrificed humanely by giving thiopental sodium (40mg/kg i.p) and brain were isolated. The different biochemicals were estimated like Glutathione (GSH), Superoxide dismutase (SOD), Lipid peroxidation (LPO), Protein estimation and observed histopathological changes of the brain.[22-23]

b) 3-Nitropropionic (3-NP) acid induced neurotoxicity model.

Total 30 rats were randomly divided into five groups of 6 rats each and treated as follows:

Table 3: 3-Nitropropionic acid (3-NP) induced neurotoxicity model:

SL NO.	Group	Treatment	Duration of treatment
1.	Negative control	vehicle +Normal saline	Control group vehicle alone for 7 days (oral) and from 8th day onward vehicle followed by normal Saline (i.p) after 2hours daily for 7 days
2.	Positive control	Normal saline + 3-NP 10 mg/kg (i.p)	Normal saline alone for 7 days (oral) and from 8th day onward saline followed by 3-NP 10 mg/kg (i.p) after 2 hours daily for 7 days.
3.	Moringa concanensis (low dose: 200mg/kg)	Moringa concanensis extract low dose (p.o) + 3-NP 10mg/kg (i.p)	Pretreatment for 7 days with Moringa concanensis extract low dose (oral) and from 8th day on ward Moringa concanensis extract followed by 3-NP 10 mg/kg (i.p.) after 2 hours daily for 7 day in test group
4.	Moringa concanensis (high dose:400mg/kg)	Moringa concanensis extract high dose (p.o) + 3-NP 10 mg/kg (i.p)	Pretreatment for 7 days with Moringa concanensis extract high dose (oral) and from 8th day on ward Moringa concanensis extract followed by 3-NP 10 mg/kg (i.p.) after 2 hours daily for 7 day in test group
5	Standard (Piracetam)	3-NP 10mg/kg (i.p) + Piracetam 200mg/kg (p.o) .	Pretreatment for 7 days with Piracetam 200mg/kg and from 8th day on ward Piracetam followed by 3-NP 10 mg/kg(i.p) after 2 hours daily for 7 day in standard group

The Moringa concanensis extract was administered before 2 hours administration of 3-NP. During the drug treatment rats will be observed for the behavioural changes for 50 minutes daily. On 14th day after 4 hours of 3-NP administration rats were evaluated for General behavioural studies such as string test for Grip strength, Locomotor activity, Rota-rod performance assessment for motor coordination, Elevated plus maze test. On 15th day rats were sacrificed humanely by giving thiopental sodium (40mg/kg i.p) and brain were isolated. The different biochemicals were estimated like Glutathione (GSH), Superoxide dismutase (SOD), Lipid peroxidation (LPO), Protein estimation and observed histopathological changes of the brain.[24-25]

Parameters monitored Measurement of body weight change

Animal body weight was noted on the first day and last day of the experimentation. Percentage change in the body weight was calculated in

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comparison to the initial body weight on the first day of the experimentation.[24]

Behavioural parameters

A. Movement analysis (Neurological changes):

Neurotoxins are associated with several motor disturbances which prevent normal ambulatory movement of the animal. Severity of the motor abnormalities in these groups was therefore evaluated using a quantitative neurological scale. A neurological score was determined for each animal on 7th day of Alcl3 and 14th day of 3NP after 4 h of last dose incomparison to control animals. (Score = 0, normal behavior; score = 1, general slowness of displacement resulting from mild hind limb impairment; score = 2, in coordination and marked gait abnormalities; score = 3, hind limb paralysis; score = 4, incapacity to move resulting from fore limb and hind limb impairment; score =5, recumbecy).[24]

B. Locomotor activity:

The spontaneous locomotor activity is monitored using actophotometer equipped with infrared sensitive photocells, the apparatus will be placed in darkened, light and sound attenuated and ventilated testing room. Before locomotor task, animals will be placed individually in the activity meter for 2 min for habituation. Therefore, locomotor activity were recorded for a periodof 10 min. The locomotor activity will be expressed in terms of total photo beam counts/ 10 min.[26]

Percentage decrease in the motor activity= (B - A/ B) X 100A = After Drug Administration
B = Before Drug Administration

C. Elevated plus maze test for special memory:

The spatial long-term memory of rats was assessed by using the EPM test. A typical EPM apparatus consists of two open arms (length 500 mm × width 100 mm) and two close arms (length 500 mm × width 100 mm × height of the side walls 400 mm). The maze was elevated to the height of 500 mm from the floor. In the middle of the maze the arms were connected by a central square . During the acquisition trial each rat was placed individually at the end ofan open arm facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms was recorded as initial transfer latency (ITL) using a stopwatch.

If the rat did not enter into one of the closed arm within 300 sec, it was pushed on the back into one enclosed arm and the transfer latency was given as 300 sec. Later the rat was allowed to explore the apparatus for 30 sec to become familiar with the maze and then returned to its home cage. The retention trial followed 24 hrs after the acquisition trial in which time it took to move from the end of open arm and re-enter into either of the closed arms was recorded as retention transfer latency (RTL) using a stopwatch.[27]

% Memory retention=(ITL- RTL)/RTL× 100

D. Rota- rod performance assessment for motor coordination:

The rota rod (rotating) test is widely used in rodents to assess their "minimal neurological deceit " such as impaired motor function (eg. Ataxia) and coordination. The Rota rod unit consists of arotating rod, 75mm in diameter, which was divided into four parts by compartmentalization to permit the testing of four rats at a time . Briefly, in a training session the rats were placed on the rod that was set to 25 rpm and the performance time that each rats were able to remain on the rota rod was recorded . The rats was subjected to three trails at 3 to 4 hours intervals on two separate days for acclimatization purposes. In the test session, the rats were placed on the rota rod and their performance was recorded.[28]

Percentage decrease in Time/ Motor Activity= (B - A/ B) X 100

E. String test for grip strength:

The rat was allowed to hold with the forepaws a steel wire (2 mm diameter and 80 cm in length), placed at a height of 50cm over a cushion support. The length of time the rat was able to hold thewire was recorded.

Histopathological study of rat brain:

A section of the brain will be fixed with 10% formalin and embedded in paraffin wax and cutinto sections of 5µm thickness. The sections will be stained with haematoxylin and eosin dye for histopathological observations. Depending on the model, either hippocampal or striatal neurons will be observed for morphological changes.[24]

Statistical evaluation:

The values observed were expressed as mean ± SEM. Statistical difference in mean was analysed using one way ANOVA followed by Dunnet's multiple comparison tests. $P < 0.05$ was considered as statistically significant.

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II. RESULT AND DISCUSSION

Acute oral toxicity studies:

The ethanolic extract did not show any sign and symptoms of toxicity and mortality up to 1600 mg/kg dose. Based on the results obtained from this study, the dose of further pharmacological studies were fixed to be 200 and 400mg/kg.[20]

Phytochemical Analysis:

The plant extract subjected for phytochemical study showed the presence of Flavonoids, Alkaloids, proteins or amino acids, cardiac glycosides, phenols, Terpenoids, carbohydrates (Table 6).

Table 5 : Quantitative Analysis of the Phytochemicals of Moringa concanensis

S. No	Phytochemical	Result
1.	Alkaloids	Present
2.	Glycosides	Present
3.	Flavonoids	Present
4.	Cardiac glycosides	Present
5.	Terpenoids	Present
6.	Proteins	Present
7.	Phenols	Present
8.	Quinones	Present
9.	Carbohydrates	Present
10.	Steroids	Present

Alcl3 induced neurotoxicity :

Table 6 : % of body weight changes in Alcl3 induced neurotoxicity in rats :

Treatment	NormalControl	Positive control	Low dose (200 mg/kg)	High dose (400 mg/kg)	Standard (200 mg/kg)
Before	201.8±4.868	206±8.046	187.8±2.25	194.2±3.092	197.3±6.771
After	210±4.320	194.3±7.868	190.7±2.15	201.8±2.496	208.2±7.002

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%Body weightchange	104	94.3***	101.5##	103.9#	105.5

Values are expressed as mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's multiple comparison test. Level of significance *** (P < 0.001) when compared to negative control rats and ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.

Table 7 : Effect of Moringa concanensis on Behavioral characters in Alcl3 induced neurotoxicity in rats.

Treatment	Neurological Score	Locomotar activity (counts/5min)	Plus maze		Rotarod performan ce (S)	Grip strength test(S)
			[Transfer latency time sec]	[%] memory retention		
Normalcontrol	0.0	617.5±7.95	16.3±0.821	74.82	30±1.48	31.6±0.975
Positivecontrol	3.03±0.042***	265.7±1.62**	21.1±0.354 ***	29.50	13.5±0.76 ***	14.5±0.676 ***
Low dose (200mg/kg)	2.41±0.011##	339.5±2.47	8.60±0.128 ###	35.70	22.30±0.89 ###	20.7±1.021 ###
High dose (400mg/kg)	1.81±0.019###	550±3.80##	10.84±0.42#	49.22	28.81±1.56 ##	28.6±0.695#
Standard (pirecetam)	0.75±0.026	605.6±3.99	11.9±0.780	71.42	31.5±1.92	29.81±0.67

Values are expressed as mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's multiple comparison test. Level of significance *** (P < 0.001), ** (P < 0.01) when compared to negative control rats and ### (P < 0.001), ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.

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Figure 14 Behavioural characters of Moringa concanensis leaves extract in $AlCl_3$ induced neurotoxicity in rats % of body weight change

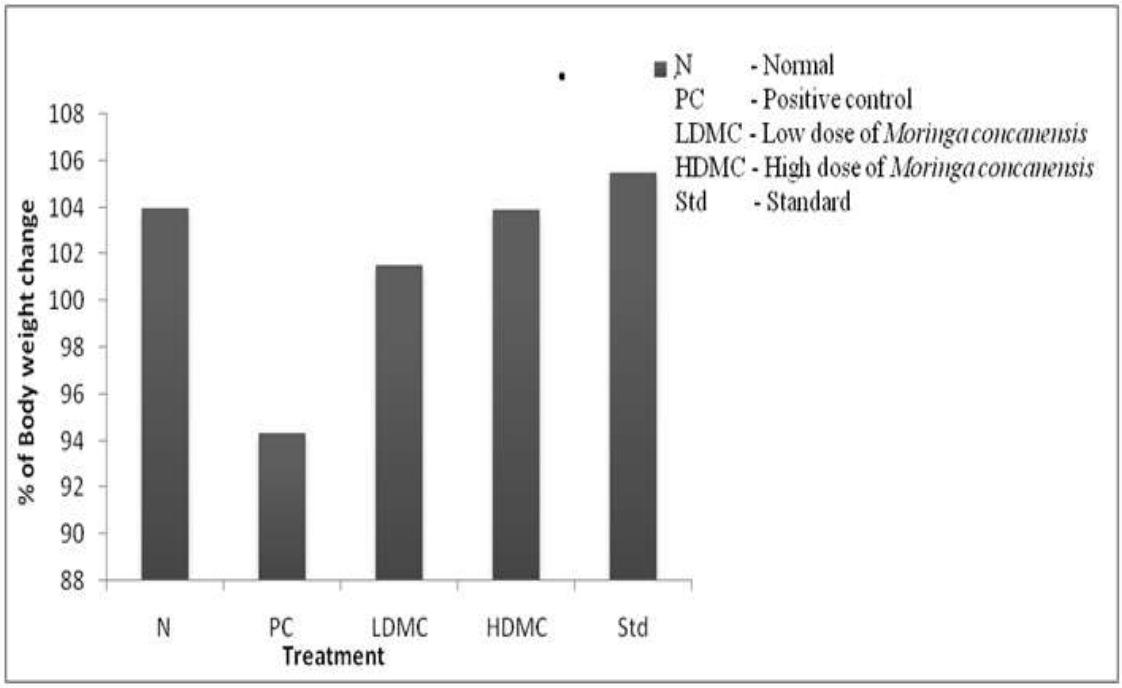

Figure 14(A): Effect of leaves extract of Moringa concanensis on % body weight change in $AlCl_3$ induced neurotoxicity

Neurological score

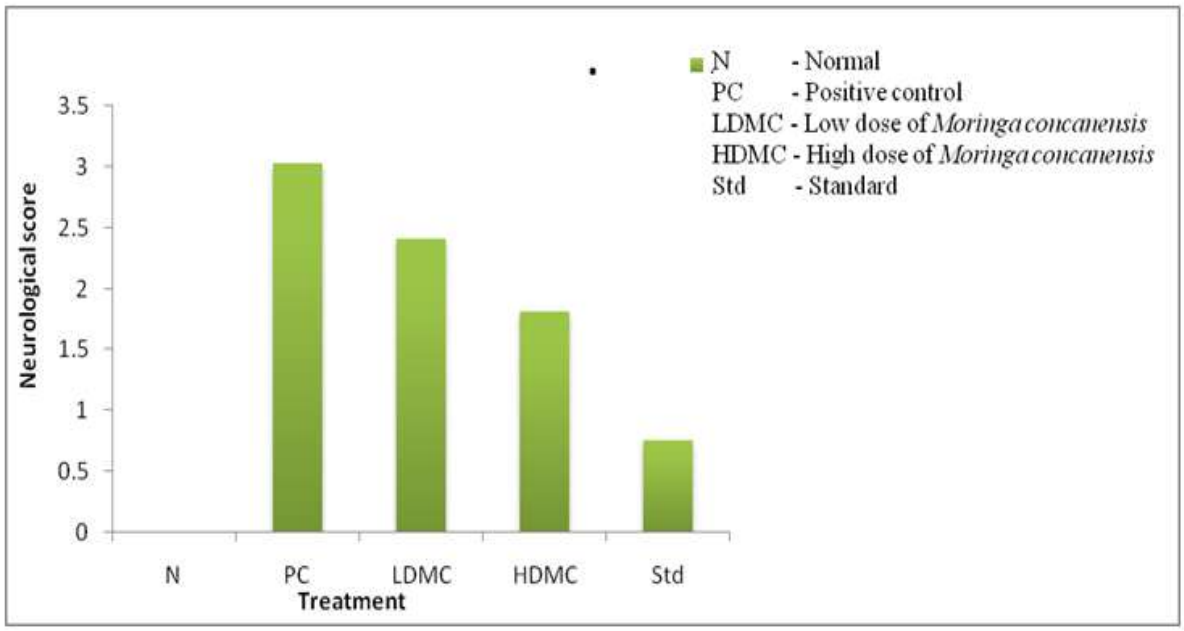

Figure 14(B) : Effect of leaves extract of Moringa concanensis on neurological source in $AlCl_3$ induced neurotoxicity.

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Locomotor activity

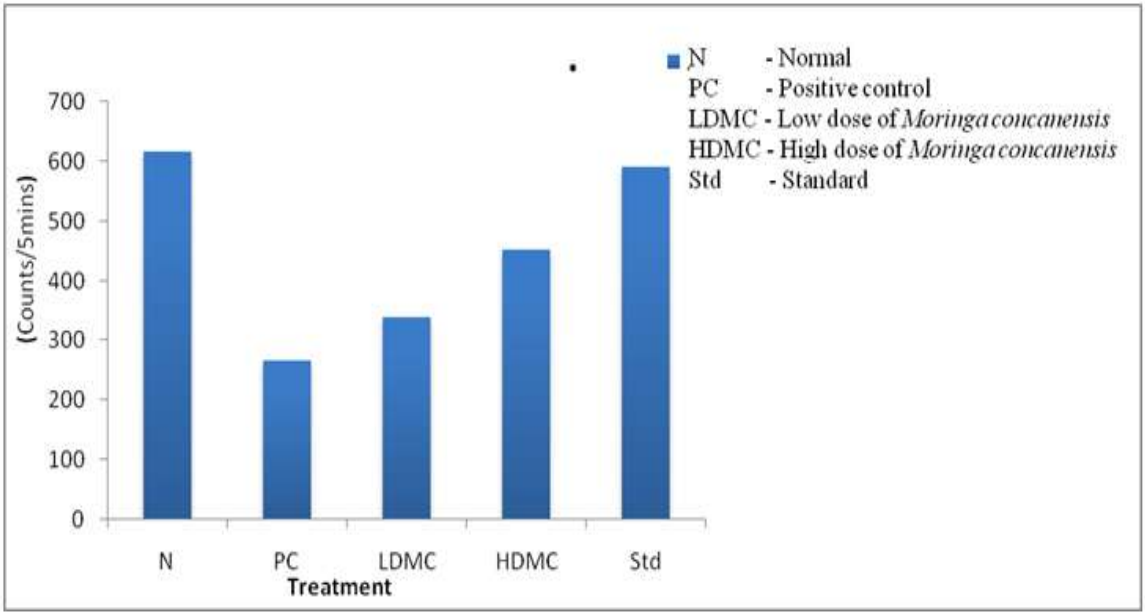

Figure 14(C) : Effect of leaves extract of Moringa concanensis on locomotor activity in$AlCl_3$ induced neurotoxicity.

Grip strength test

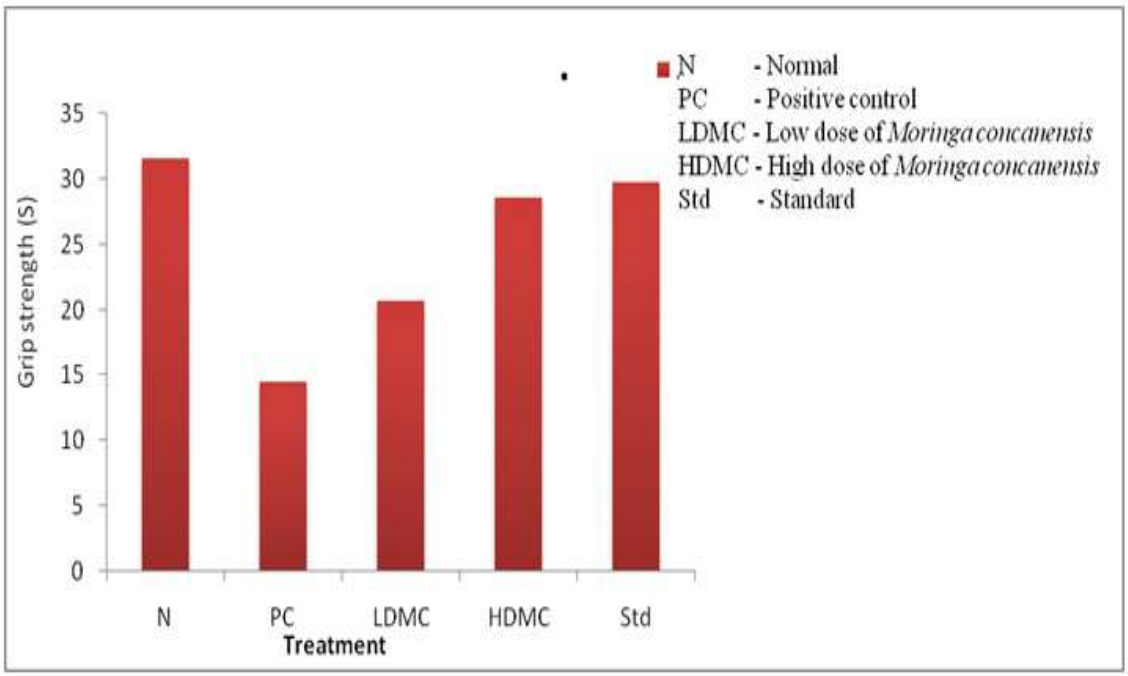

Figure 14(D) : Effect of leaves extract of Moringa concanensis on grip strength test in$AlCl_3$ induced neurotoxicity.

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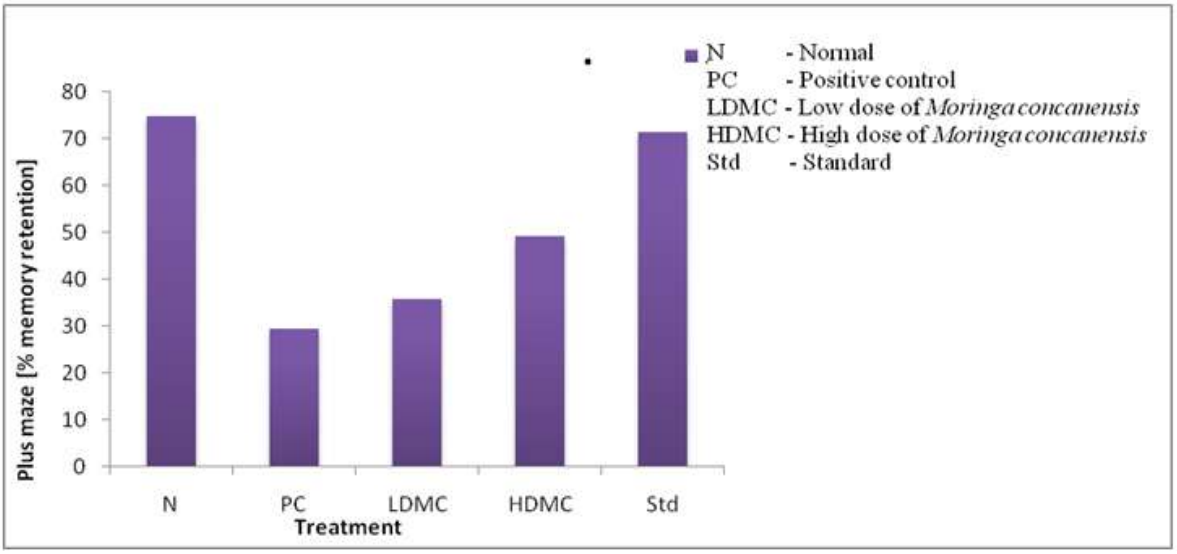
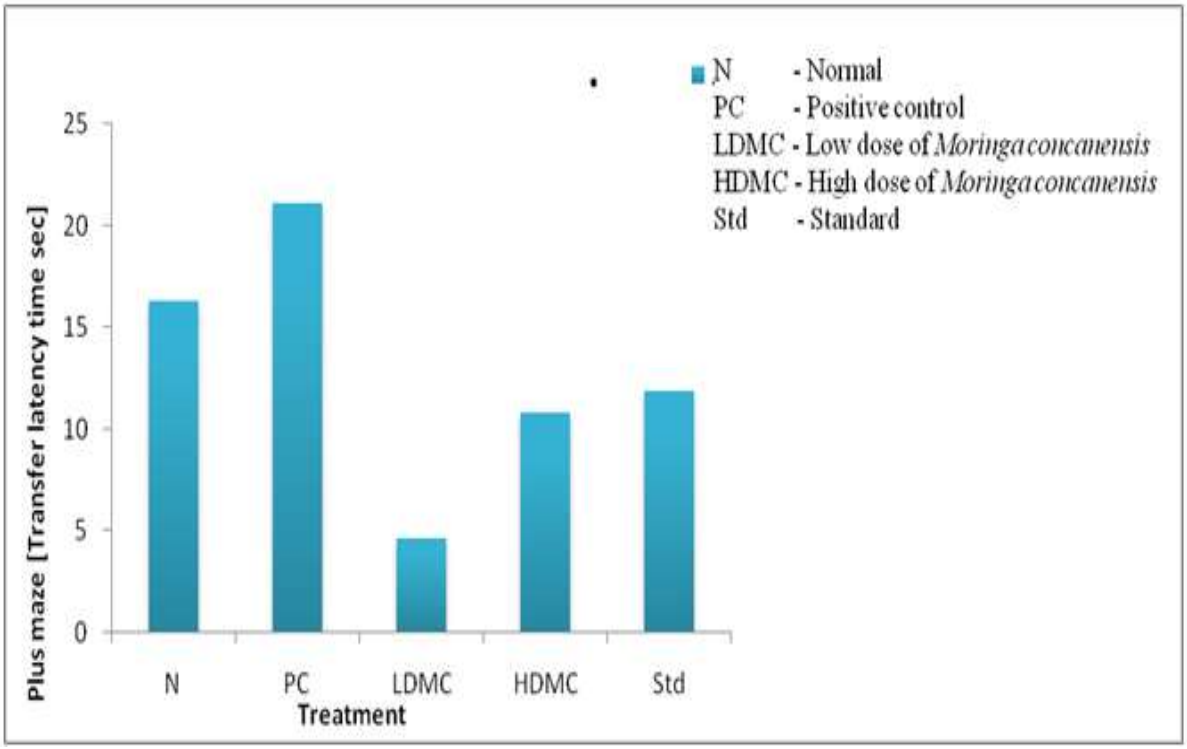
Elevated plus maze



[A]



[B]

Figure 14(E) : Effect of leaves extract of Moringa concanensis on Elevated plus mazetest [A , B] in AlCl3 induced neurotoxicity.

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Rota rod performance

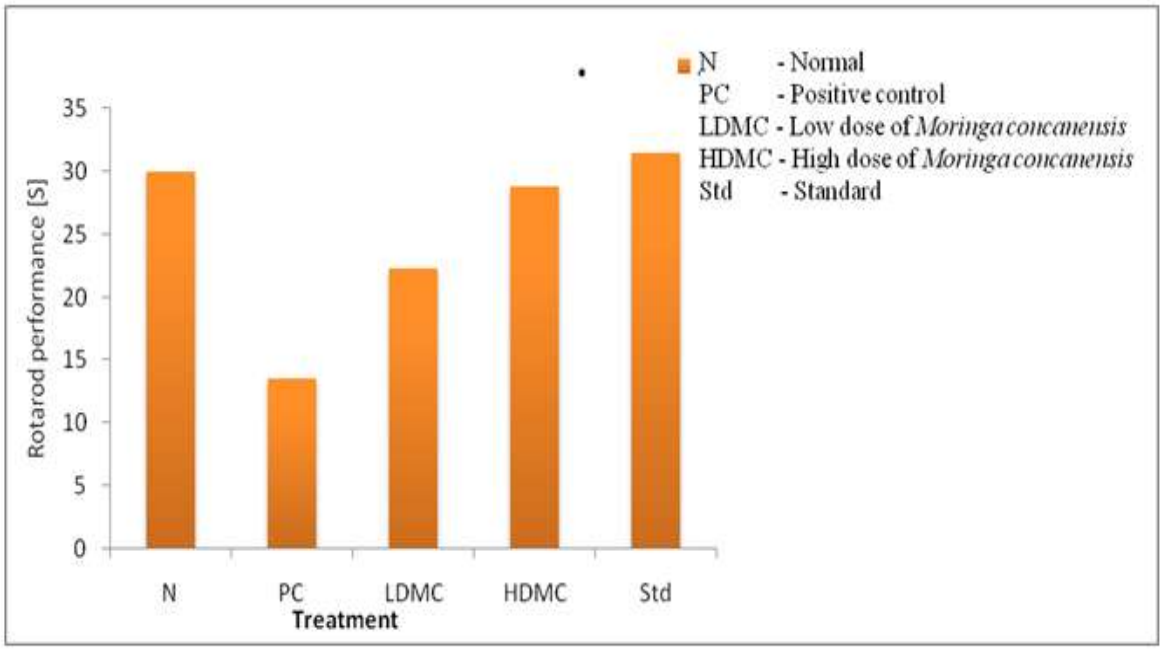

Figure 14(F) : Effect of leaves extract of Moringa concanensis on rota rod performance in$AlCl_3$ induced neurotoxicity.

Table 8 : Effect of Moringa concanensis extraction antioxidant levels in Alcl3 inducedneurotoxicity in rats.

Treatment	Lipid peroxidation (nmoles MDA/g of protein)	Reduced glutathione (nmoles/min/mg of protein)	Super oxide dismutase (units/min/mg of protein)	Protein estimation (g/dl of total protein
Normal control	232.7±1.28	1.81 ±0.072	20.9±1.04	8.18±0.29
Positive control	543.6±1.87***	0.754±0.037***	10.72±0.91***	3.6±0.28***
Low dose (200mg/kg)	### 357.4±1.92	### 1.181±0.046	## 13.38±0.66	## 6.89±0.17
High dose (400mg/kg)	## 256.5±2.04	### 1.36±0.042	15.96±1.054	# 7.64 ±0.36
Standard	248.26±2.95	1.51±0.022	17.83±1.05	7.76±0.17

Values are expressed as mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's multiple comparison test. Level of significance *** (P < 0.001) when compared to negative control rats and ### (P < 0.001), ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.

Histopathology

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Effect of Alcl3 induce neurotoxicity on histopathological analysis of Rat brain.

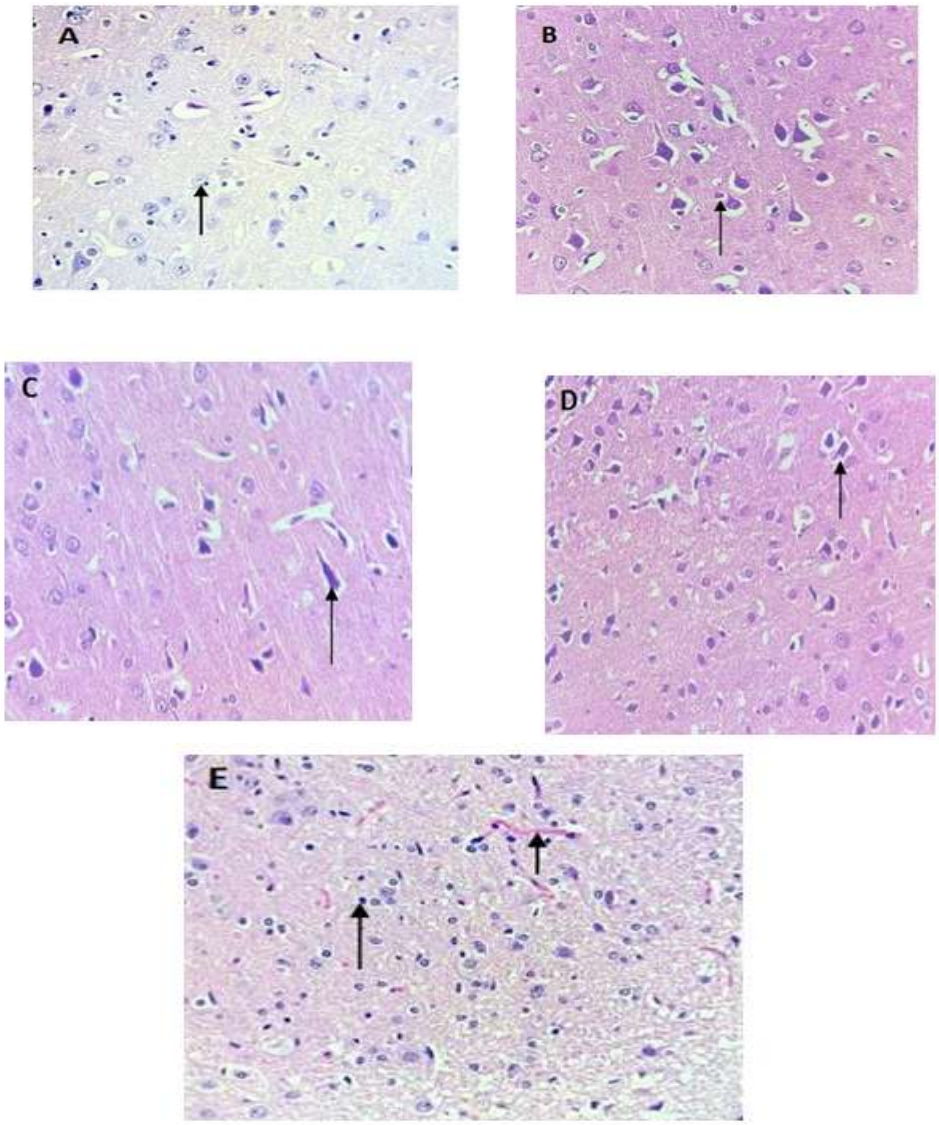

Figure 16: The brain section stained by Hematoxylin-Eosin and observed (Magnification 400x) histological structure of cerebral cortex region. **A:** Normal control **B:** Positive control (Alcl3 50mg/kg), **C:** Low dose (200mg/kg + Alcl3 50mg/kg), **D:** High dose (400mg/kg + Alcl3 50mg/kg), **E:** Standard (Piracetam 200mg/kg + Alcl3 50mg/kg).

3-NP induced neurotoxicity :
Monitored Parameters:
Body weight changes in 3NP induced neurotoxicity in rats :

Table 9 : % of body weight changes in 3NP induced neurotoxicity in rats

Treatment	NormalControl	Positive control	Low dose (200 mg/kg)	High dose (400 mg/kg)	Standard (200 mg/kg)
Before	193.7±3.86	198.36±4.39	194.16±4.40	199±4.36	202±4.62

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After	201.26±3.24	191.25±3.38	192.3±4.38	202.8±4.27	213.2±4.77
% Body weight change	103.9	96.4***	99.04##	101.90#	105.5

Values are expressed as mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's multiple comparison test. Level of significance *** (P < 0.001) when compared to negative control rats and ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.

Table 10 : Effect of Moringa concanensis on Behavioral characters in 3NP inducedneurotoxicity in rats.

Treatment	NeurologicalScore	Locomotar activity (counts/5min)	Plus maze (transfer latency time sec)		Rotarod performance(S)	Grip strength test (S)
			[transfer latency time sec]	[%] memory retention		
Normal control	0.0	520.3±1.50	11.4±0.74	78.7	33.26±1.84*	31.33±1.43
Positive control	3.04±0.26***	247±2.9***	25.5±0.54***	37.9	18.4±1.77***	15.26±0.56***
Low dose (200mg/kg)	2.13±0.288# ##	360.4±2.62 ###	17.9±0.93##	48.5	## 25.22±0.96	21±0.97 ###
High dose (400mg/kg)	# 1.53±0.334	525.5±2.34 #	16.1±0.96#	62.6	28.53±0.70#	28.13±1.02#
Standard (pirecetam)	1.49±0.194	527.6±3.97	13.74±0.85	71.3	29.93±1.08	30.4±1.04

Values are expressed as mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's multiple comparison test. Level of significance *** (P < 0.001), when compared to negative control rats and ### (P < 0.001), ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.

Figure 17 - Behavioural characters in 3-NP induced neurotoxicity in ratsBody weight change

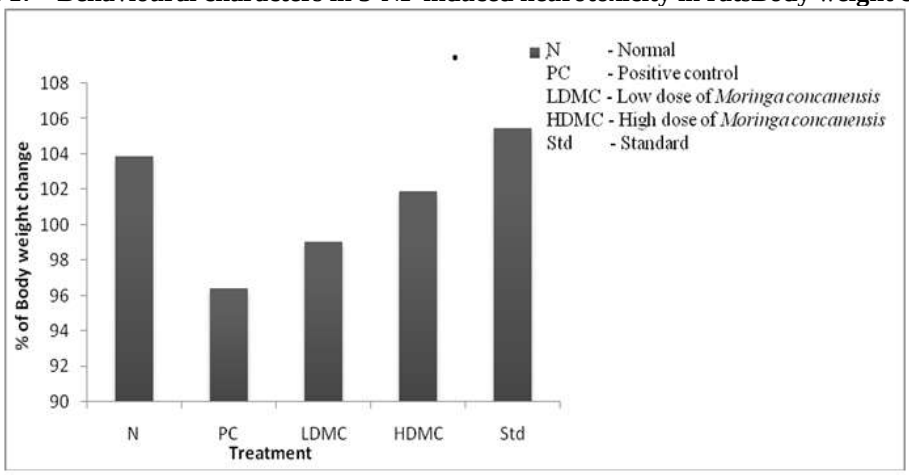

Figure 17(A) : Effect of leaves extract of Moringa concanensis on % body weight changein 3-NP induced neurotoxicity

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Neurological score

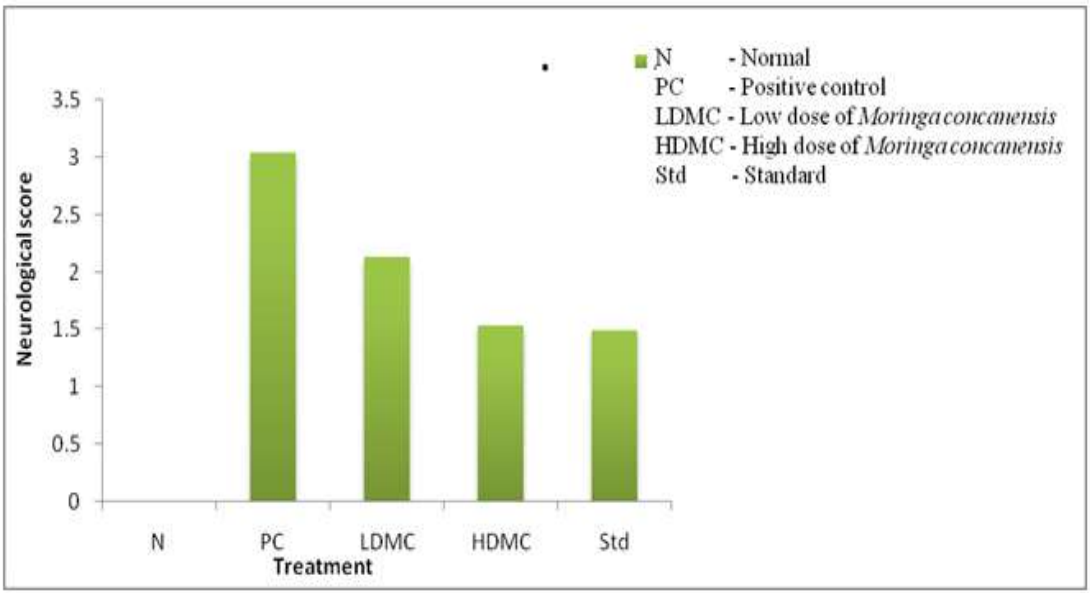

Figure 17(B) : Effect of leaves extract of Moringa concanensis on neurological source in3-NP induced neurotoxicity.

Locomotor activity

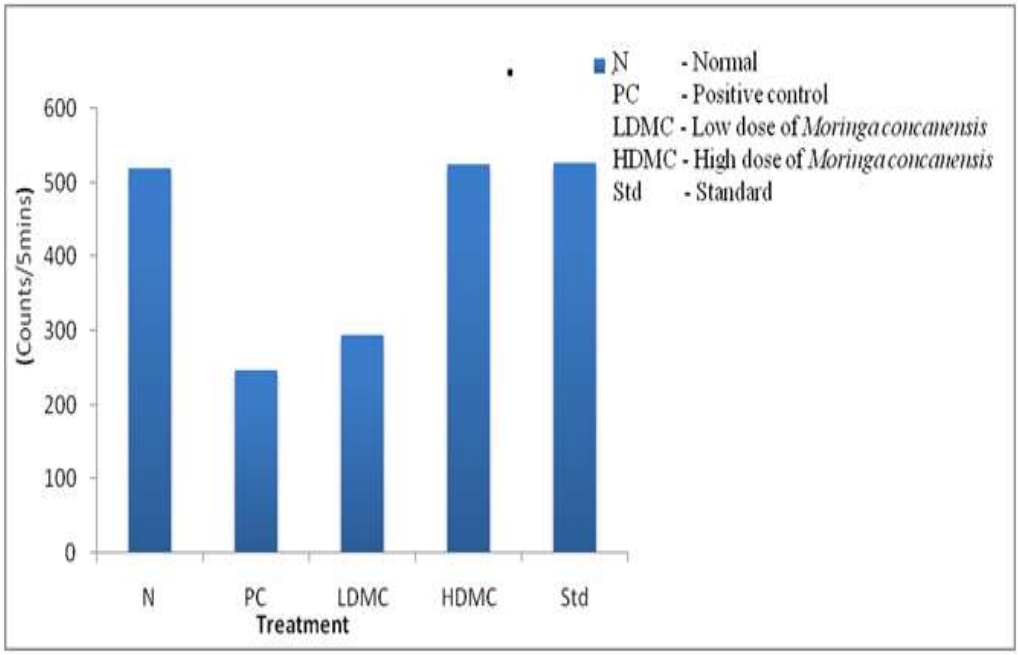

Figure 17(C) : Effect of leaves extract of Moringa concanensis on locomotor activity in3-NP induced neurotoxicity.

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Grip strength test

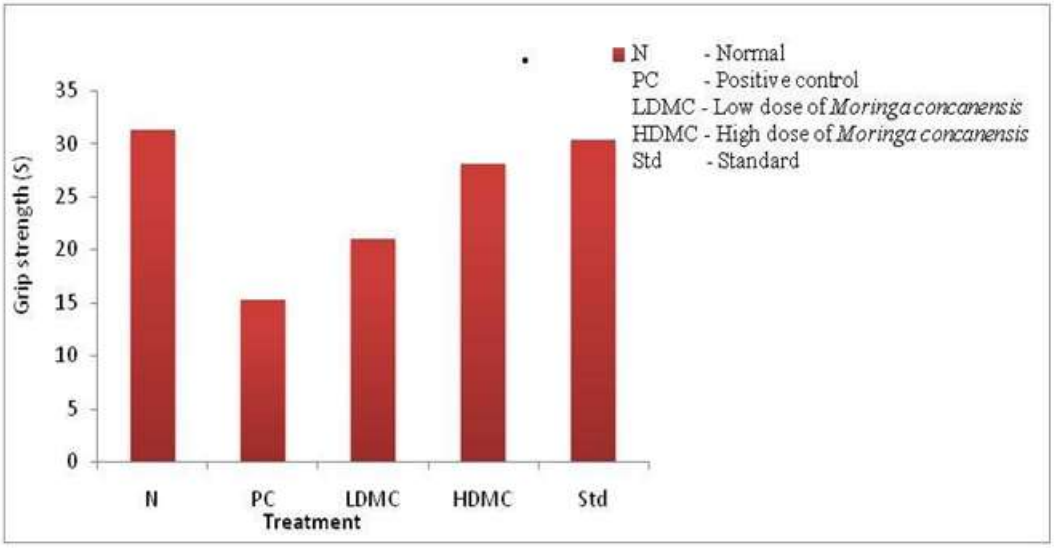
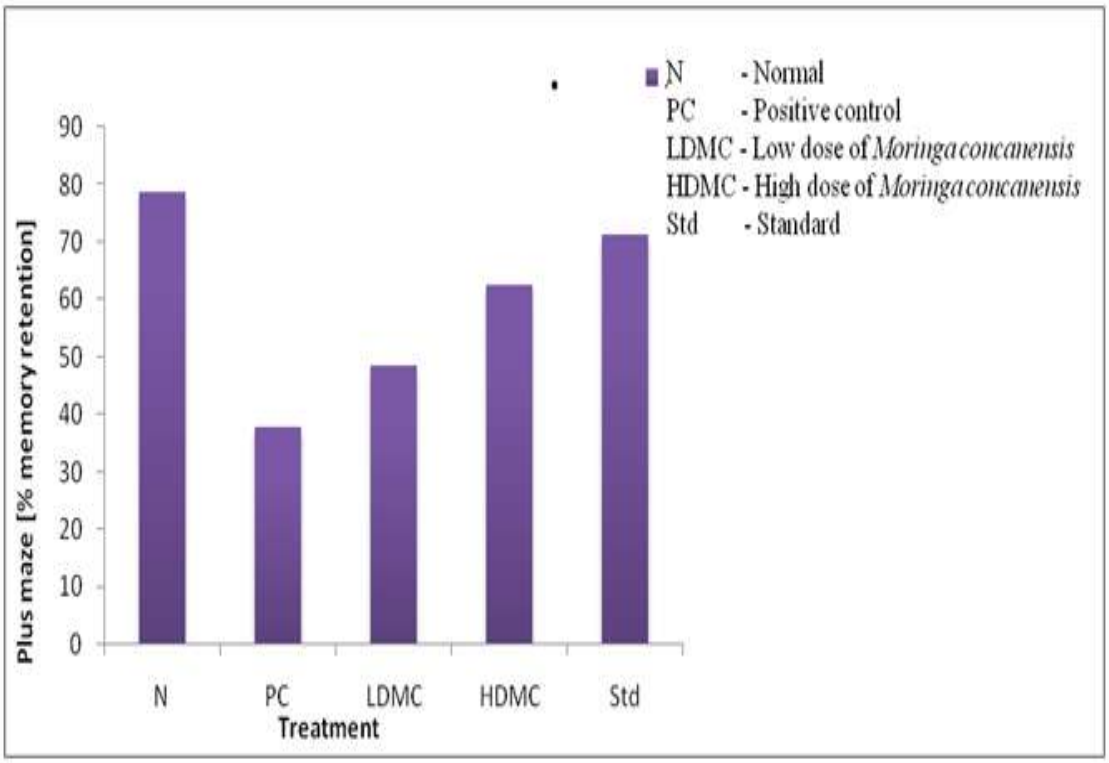


Figure 17(D): Effect of leaves extract of Moringa concanensis on grip strength test in 3-NPinduced neurotoxicity.

Elevated plus maze



[A]

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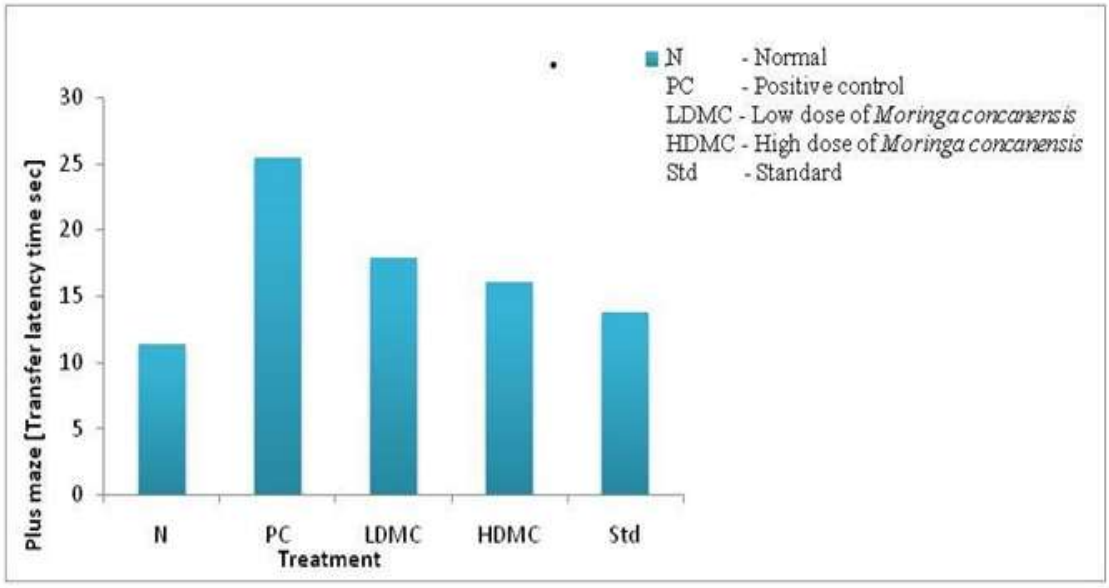

[B]

Figure 17(E) : Effect of leaves extract of Moringa concanensis on Elevated plus mazetest [A , B] in 3-NP induced neurotoxicity.

Rota rod performance

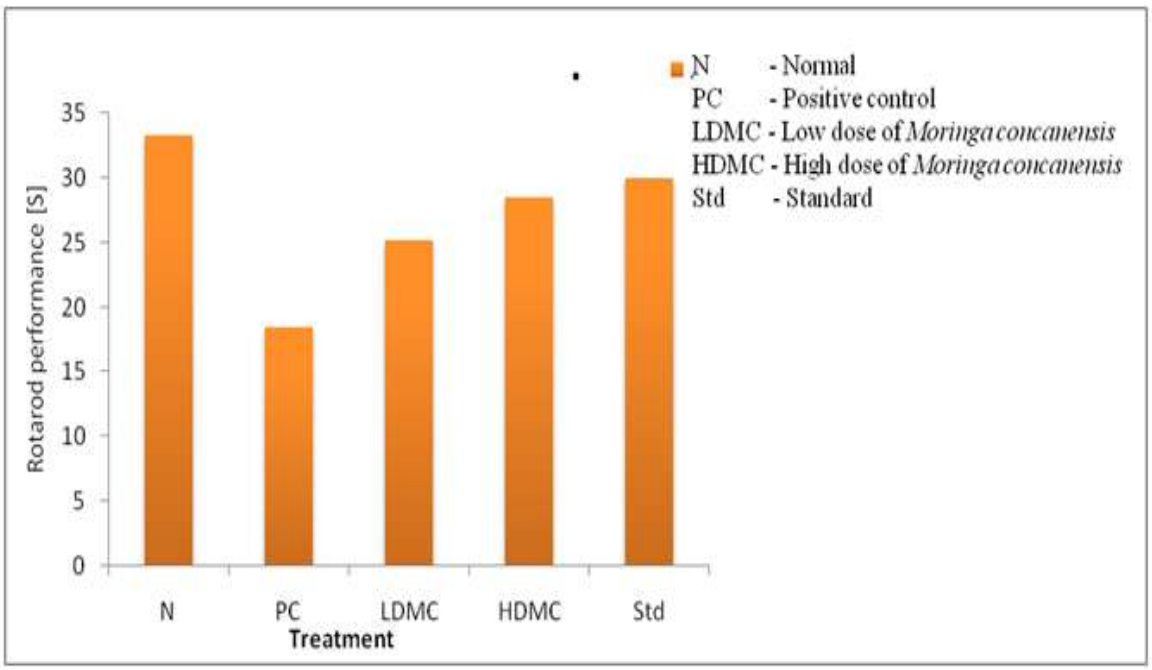

Figure 17(F) : Effect of leaves extract of Moringa concanensis on rota rod performance in3-NP induced neurotoxicity.

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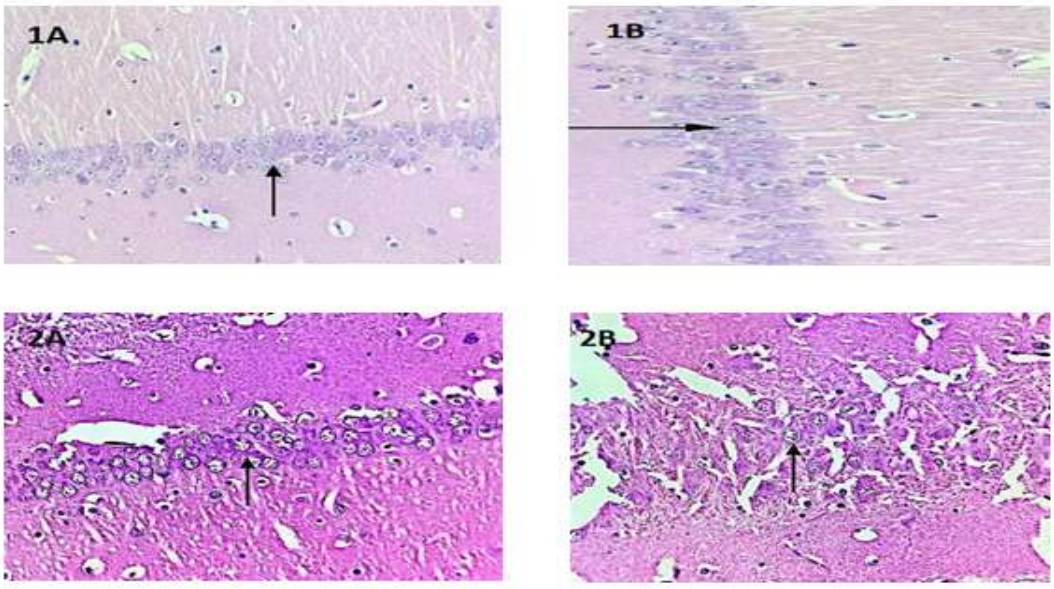
Table 11: Effect of Moringa concanensis extraction antioxidant levels in 3 NP induced neurotoxicity in rats:

Treatment	Lipid peroxidation (nmoles MDA/g of protein)	Reduced glutathione (nmoles/min/mg of protein)	Super oxide dismutase (units/min/mg of protein)	Protein estimation (g/dl of total protein
Normal control	163.26±4.16	1.73 ±0.015	19.75±0.61	9.36±0.58
Positive control	600.4±6.55***	0.862±0.040***	10.16±0.22***	4.15±0.11***
Low dose (200mg/kg)	### 360.6±6.03	### 1.152±0.032	### 12.87±0.94	7.83±0.25
High dose (400mg/kg)	### 277.4±1.83	## 1.69±0.047	## 16.75±0.99	8.26 ±0.27#
Standard	208.81±3.12	1.83±0.022	19.96±0.87	8.89±0.62

Values are expressed as mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's multiple comparison test. Level of significance *** (P < 0.001), when compared to negative control rats and ### (P < 0.001), ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.

Effect of 3-NP induce neurotoxicity on histopathological analysis of Rat brain.



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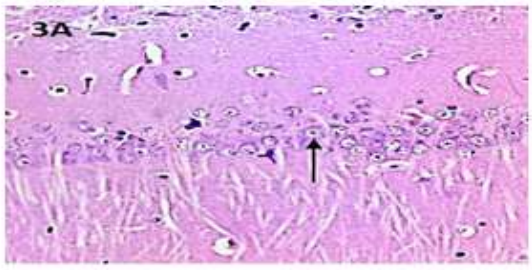

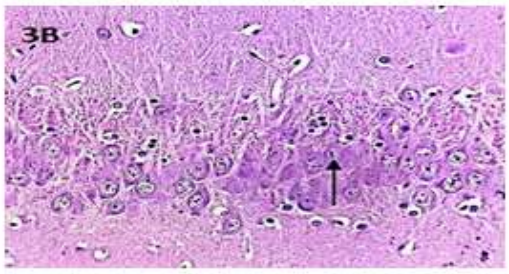

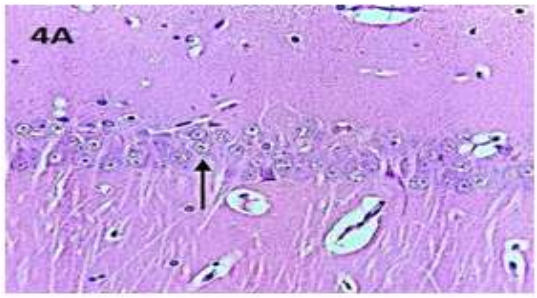

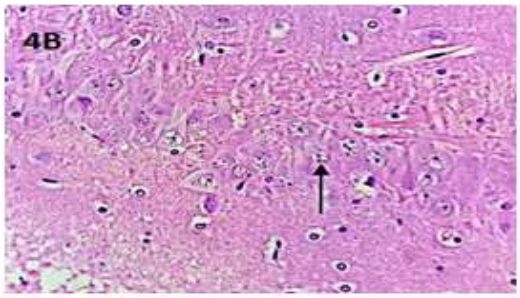

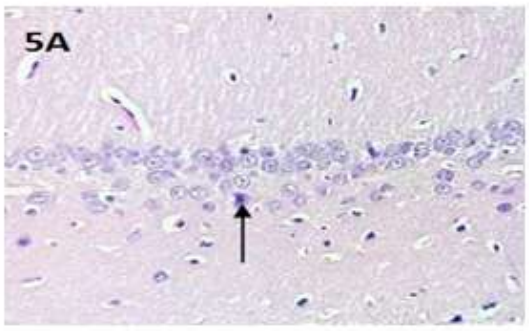

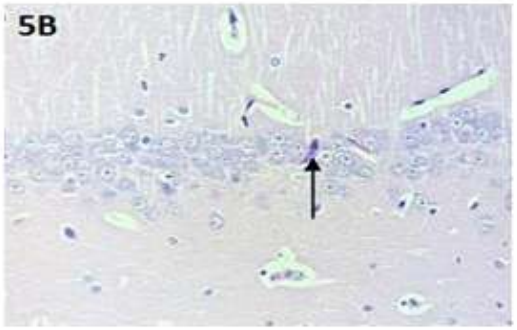

- In conclusion, The ethanolic leaves extract of Moringa concanensis significantly attenuated behavioural alterations, oxidative damage, mitochondrial dysfunction, striatal/hippocampus damage in 3-Nitropropionic acid neurotoxicity and prevented the increase in activity of acetyl-cholinesterase induced by $AlCl_3$ in the cortex hippocampus and striatum.
- The results of the current study reveal that the Neuroprotective activity demonstrated by M c plant extract might be due to the presence of phytoconstituents, such as Flavonoids, alkaloids, tannins, saponins, phenol and terpenoids.
- The –study also showed that there was a reversal of the brain damage in animals treated with leave extract of Moringa concanensis and it also prevented the neuron loss.
- Therefore this result clearly indicates that administration of Moringa concanensis leaves extract have potent therapeutic neuro protective activity which indicate through behavioral parameters.

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