AQUEOUS EXTRACT OF MORINGA OLEIFERA PROMOTE DEVELOPING CEREBRUM OF WISTAR RATS

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ABSTRACT

Herbal medicine is gaining popularity worldwide due to its efficacy, cost effectiveness and minimal side effects. Moringa oleifera is a plant whose parts are used for various purposes ranging from medicinal, horticultural and human consumption. This work was carried out to know some effects of Moringa oleifera on the histology of the developing cerebrum of Wistar rats, average weight of the animals and relative brain weight. Fifteen (15) Wistar rats of both sexes of average weight between 20-30 g of about 3 weeks old were divided into three groups of 5 animals each. Group 1 was the control group and received 2mls of distilled water each. Group 2 received 100mg/kg of the extract, while Group 3 received 200mg/kg of the extract. The experiment was done for 6 weeks. After 6 weeks the animals were sacrificed using cervical dislocation. The cerebrum were removed, weighed and fixed in formol-calcium for H&E staining. The results showed that feed intake of the animals in group 2 was significantly different

compared to the control group from the 4th week of administration, while group 3 animals feed intake was significantly different from control throughout the six weeks . There was no significant difference in the relative brain weight and the mean weight of animals across the group. Histological studies showed the aqueous extract of moringa oleifera had only a minimal effect on the micro-architecture of the prefrontal cortex of wistar rats. This study revealed that moringa has a neuro-protective effect which might be due to the presence of protein, vitamins, minerals, beta carotene, amino acids and various phenolics.

INTRODUCTION

In the last few decades, there has been an increased growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and minimal side effects (Umer *et al*., 2002). Many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Umer *et al*., 2002). A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparation of indian traditional health care systems (Sogbo, 2006). Moringa oleifera, is the most widely cultivated species of the genus Moringa, which is the only genus in the family Moringaceae (USDA GRIN Taxonomy). English common names include moringa, and drumstick tree, from the appearance of the long, slender, triangular seed pods, horseradish tree, from the taste of the roots which resembles horseradish, or ben oil tree, from the oil derived from the seeds (USDA GRIN taxonomy). The tree itself is rather slender, with drooping branches that grow to approximately 10m in height (parotta, 2013). In cultivation, it is often cut back annually to 1–2 meters and allowed to regrow so the pods and leaves remain within arm's reach (parotta, 2013). In developing countries, moringa has potential to improve nutrition, boost food security, foster rural development, and support sustainable land care (National Research Council, 2006). It may be used as forage for livestock, a micronutrient liquid, a natural anthelmintic and possible adjuvant (Makkar *et al*., 2007) The moringa tree is grown mainly in semiarid, tropical, and subtropical areas, corresponding in the United States to USDA hardiness zones 9 and 10(USDA GRIN Taxonomy). While it grows best in dry, sandysoil, it tolerates poor soil, including coastal areas (USDA GRIN Taxonomy). It is a fast-growing, drought-resistant tree that is native to the southern foothills of the Himalayas in North-Western India (USDA GRIN Taxonomy).

As of 2010, cultivation in Hawai'i, for commercial distribution in the United States, is in its early stages. "India is the largest producer of moringa, with an annual production of 1.1 to 1.3 million tonnes of tender fruits from an area of 380 km² (sing, 2011). Among the states, Andhra Pradesh leads in both area and pro duction (156.65 km²) followed by Karnataka (102.8 km²) and Tamil Nadu (74.08 km²) (sing, 2011). In other states, it occupies an area of 46.13 km². Tamil Nadu is the pioneering state in so much as it has varied genotypes from diversified geographical areas and introductions from Sri Lanka (Rajangam *et al*., 2001) Moringa is grown in home gardens and as living fences in Tamil Nadu Southern India and Thailand, where it is commonly sold in local markets (Food and agriculture organization of the united nations, 1999).

In the Philippines, it is commonly grown for its leaves which are used in soup. Moringa is also actively cultivated by the World Vegetable Center in Taiwan, a center for vegetable research with a mission to reduce poverty and malnutrition in developing countries through improved production and consumption of vegetables. Tamil Nadu Southern India has moringa in its folk

stories and use in home gardens. It is widely cultivatedin Africa, Cambodia, Nepal, Indonesia, Malaysia, Mexico, Central and South America, and Sri Lanka (Mahajan, 2007). In some regions, the young seed pods are most commonly eaten, while in others, the leaves are the most commonly used part of the plant. The flowers are edible when cooked and are said to taste like mushrooms. The bark, sap, roots, leaves, seeds, oil, and flowers are used in traditional medicine in several countries. In Jamaica, the sap is used for a blue dye.

MATERIALS AND METHOD

Animal Model

Fifteen (15) young Wistar rats of average weight between 20g-30g were obtained and housed in standard cage under standard condition. They were fed with pellet standard rat diet and water and were kept in the animal house.

Plant Material

The Moringa Oleifera leaf was obtained in Ogbomosho, Oyo State, Nigeria. The leaf was identified and authenticated at the botany department of Pure and Applied Biology of Ladoke Akintola University of Technology. The plant material was dried for Four weeks, pulverized and grinded to achieve a fine powder form. The powdery form was taken to the department of Food science and Engineering of Ladoke Akintola University of Technology where extraction was carried out. The Moringa powder was soaked in distilled water for 24hrs, after which it was evaporated at temperature of 105˚C. The extract is then mixed with distilled water and used for administration.

Experimental Design

After the rats were procured, they were weighed and acclimatized for two weeks; Group 1 comprises of five (5) wistar rats and serves as control group and they were fed with standard diet and distilled water. Group 2 comprises of five (5) wistar rats and serves as the group given 100mg/kg/bw of aqueous leaf extract moringa and also fed with pellet and water. Group 3 comprises of five(5) wistar rats and serves as the group given 200mg/kg/bw of aqueous leaf extract moringa and also fed with pellet and water.

Administration

The route of administration was orally by using the oral cannula and the administration of moringa Oleifera took six (6) weeks.

Histological Technique

Fixation: The harvested lungs were fixed immediately in 10% formol saline. The purpose of fixation is to preserve tissues permanently in as life-like a state as possible (Weiss et al., 2010). The lungs were fully immersed into the 10% formol saline as soon as possible after the removal of the lungs to prevent autolysis.

Dehydration : After fixation, the lungs were removed and subjected to dehydration. The tissues were dehydrated in ascending grades of alcohol as follows:

70% of alcohol – 1 hr, 80% of alcohol – 1 hr, 90% of alcohol – 1 hr, 95% of alcohol – 1 hr, 99% of alcohol – 1 hr, Absolute alcohol -1 hr.

Clearing: The next step is called "clearing" and involves removal of alcohol. The tissues were cleared using xylene1&2 (for 15 minutes each) as one of clearing agents to clear away the alcohol.

Infiltration/Impregnation: The tissues were impregnated with molten paraffin wax. This was to make the tissue hard and water resistant (Wells, 1988). The above process was performed twice for 1 hour each using the automated tissue processing machine, although they can be perform manually.

Embedding: This is a process by which liquid paraffin wax is use to solidify the tissues in the embedding moulds by the use of plastic embedding cassette with a pair of warm forceps so as to give a desired result. The tissue blocks were removed and air dried until the wax becomes solidified.

Sectioning: The tissue was cut into various thicknesses from 5 to 10 microns with the use of rotary microtome. But before sectioning, the tissue blocks was properly placed into a block holder of the microtome and trimmed to expose the surface of the tissue (Presnell *et al*, 1997).

Floating: The cut section was gently lowered using fine forceps into the surface of warm water in a water bath at a regulated temperature. When the section is fully expanded and flattened, clean, grease – free slide was dipped obliquely into the water bath to pick up the section. The slides was labeled with a diamond pencil and transferred into an incubator for the slide to completely dry.

Staining: This was done using hematoxylin and eosin. After taking the section to water in order to dehydrate them, section were stained regressively in hematoxylin for 5 minute. Excess hematoxylin was removed using 1% acid alcohol consequentlyremoving its blue color which was retrieved by blueing in slow running water. Eosin was used to counterstain the sections

Staining Procedure for Hematoxylin and Eosin (H&E): The tissue section was de-waxed by immersing the slides in xylene for 2-3 minutes. After being de-waxed, the slides were immersed into a descending grades of alcohol (2 changes of absolute alcohol, 90% and 70% alcohol) and finally in a distilled water. The slides were stained in Hematoxylin, The slides were washed in running tap water for 5 – 10 minutes,The slides were then stained in Eosin for 1 – 2 minutes, The slides were dehydrated in ascending grades of alcohol,After removing from absolute alcohol, the slides were cleared in xylene.

Mounting: The slides were mounted in Dibutylpthalate xylene (DPX) and covered slipped.

Statistical Analysis

Data were analyzed using Excel 2007. Data were expressed as mean +/- standard error of the mean (Mean +/- SEM). Mean values were compared using one way analysis of variance (ANOVA). P value less than 0.05 ($P<0.05$) were taken to be statistically significant. All graph were drawn with Excel 2007.

RESULTS

HISTOLOGICAL ANALYSIS

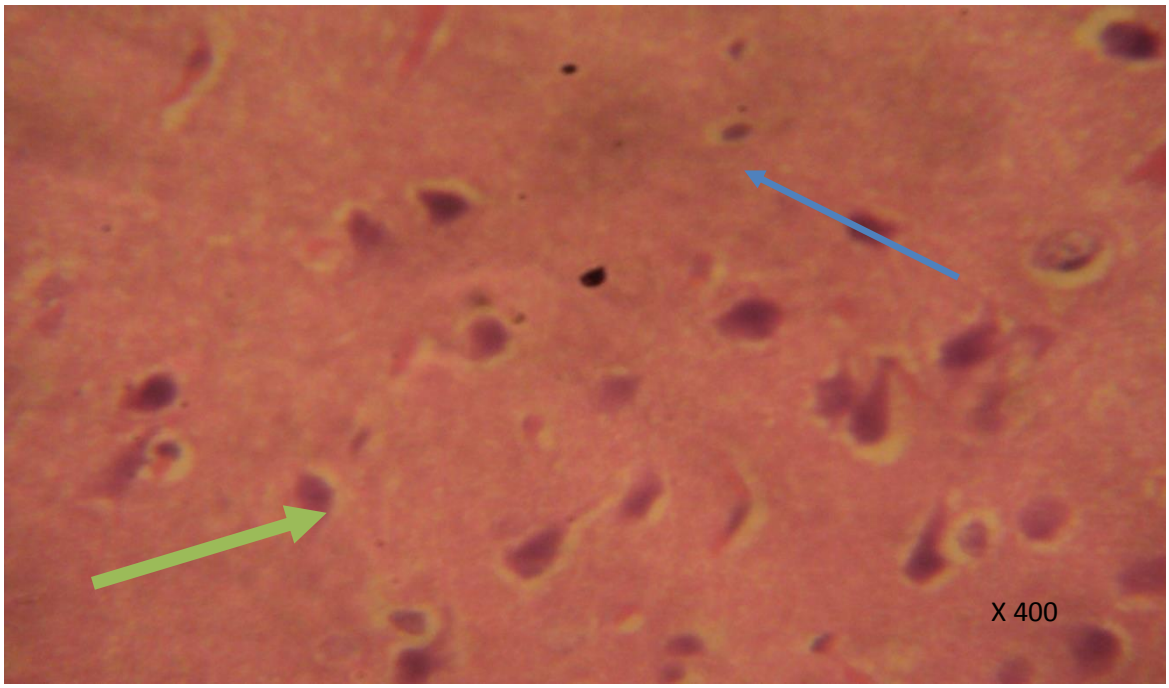


PLATE A: Photomicrograph of control section of prefrontal cortex - the control notes normal evenly distributed pyramidal neurons (Green arrow), normal perivascular space and glial cells (blue arrow).

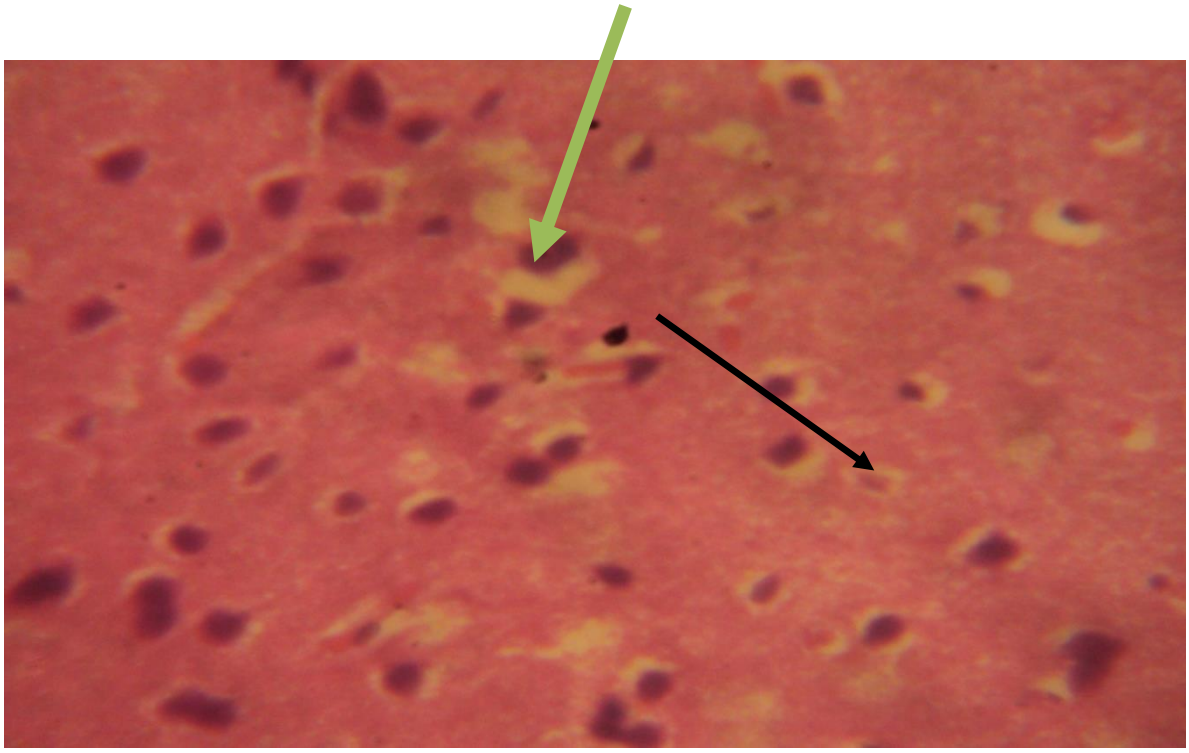
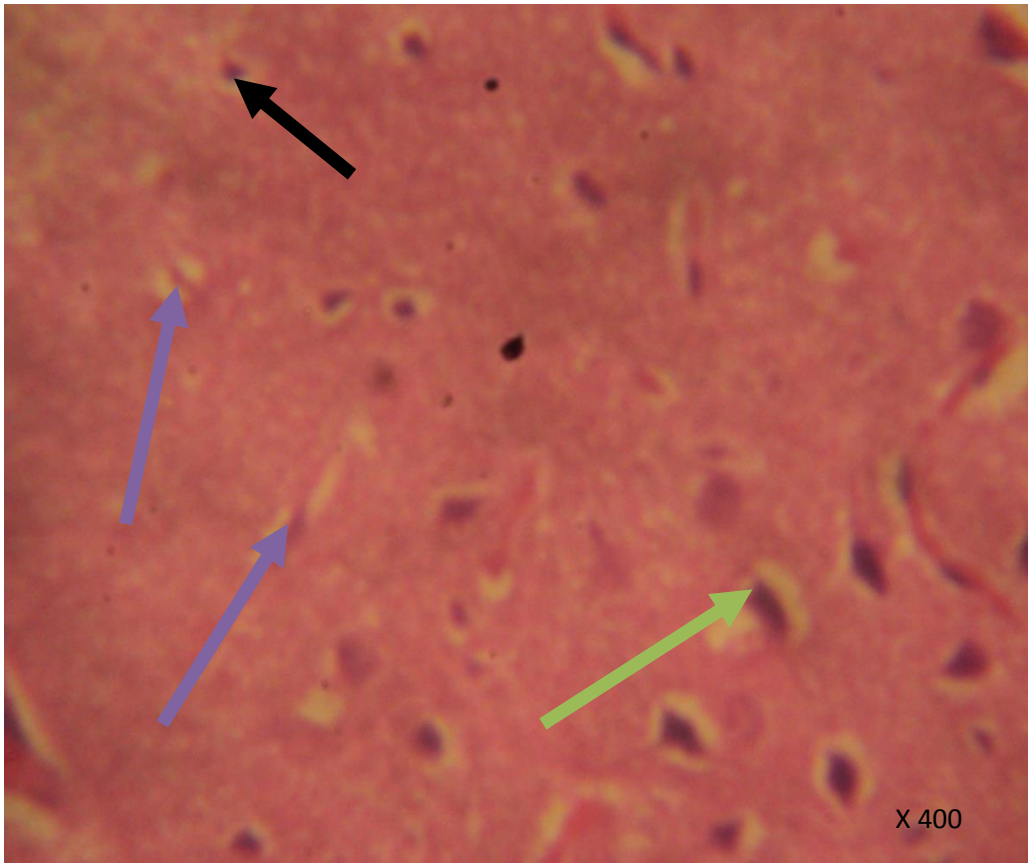


PLATE B: Light photomicrograph of sections from the prefrontal cortex of the animals administered with 100mg/kg of moringa shows normal evenly distributed pyramidal neurons(green arrow), enlarged perivascular space and scanty glial cells(black arrow).



PLATEC: Light photomicrograph of sections from the prefrontal cortex of the animals administered with 200mg/kg of moringa extract showed scanty pyramidal neurons(green arrow), pyknotic pyramidal neurons (purple arrow) and glial cells appears normal(black arrow)

TABLE 1: Shows the Mean ± SEM of the feed intake of animals, P-value of each group.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group 1	62.40 ± 0.13	80.40 ± 0.13	81.00 ± 0.71	102.8 ± 0.06	132.0 ± 0.71	123.6 ± 0.11
Group 2	62.20 ± 0.70	61.60 ± 0.06	83.00 ± 1.00	106.8 ± 0.14	137.4 ± 0.14	157.0 ± 0.89
Grp 1vs 2 p-value	0.7853	<0.0001*	0.1411	<0.0001*	<0.0001*	<0.0001*
Group 3	59.00 ± 0.32	77.00 ± 0.71	85.00 ± 0.63	106.0 ± 0.63	136.0 ± 1.3	164.4 ± 0.14
SGrp 1vs 3 p-value	<0.0001*	0.0015*	0.0029*	0.0010*	0.0272*	<0.0001*

- P-value significant difference (<0.05)

The results showed that feed intake of the animals in group 2 was significantly different compared to the control group from the 4th week of administration, while group 3 animals feed intake was significantly different from control throughout the six weeks of administration.

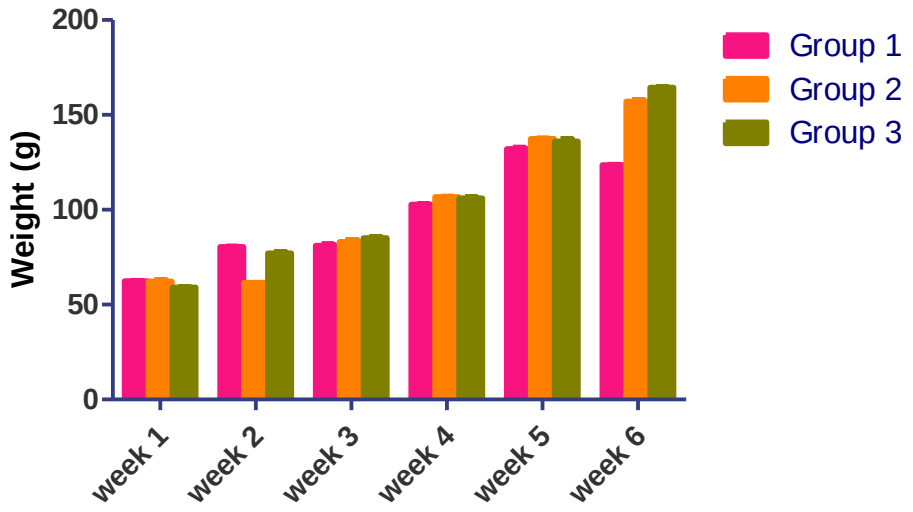


Figure 2: Graphical representation of the feed intake of the animals.

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RELATIVE BRAIN WEIGHT

Table 2: shows the relative brain weight of the animals.

	RELATIVE BRAIN WEIGHT (%)
Group 1	2.150 ± 0.1300
Group 2	1.864 ± 0.1654 0.2111
Group 3	2.082 ± 0.1111 0.7012

*P-value significant difference (<0.05)

The result shows that there was no significant difference in the relative brain weight of animals in group 2 and 3 compared to the control.

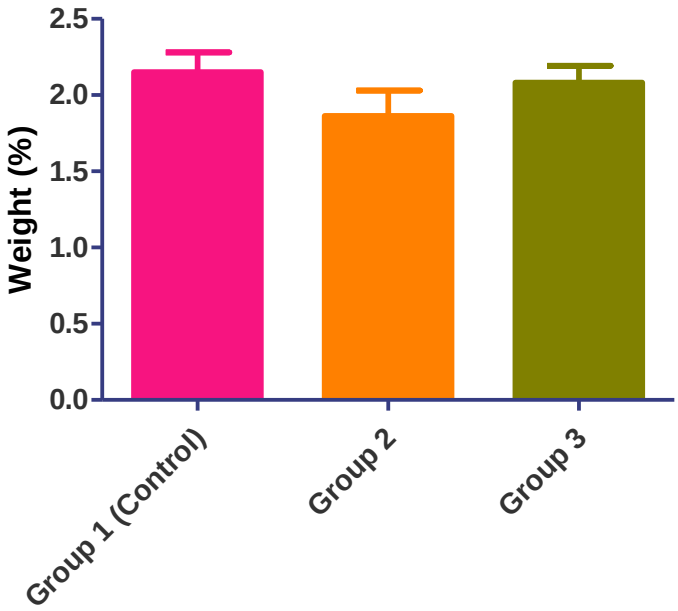
2.5
2.0
1.5
1.0
0.5
0.0
Weight (%)
Group 1 (Control)
Group 2
Group 3

Figure3: Graphical representation of the relative brain weight of animals

MEAN WEIGHT ±SEM TABLE

Table 3: Shows the Mean weight ± SEM of the animals

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group 1 (control)	22.00 ± 2.00	40.00 ± 4.47	50.00 ± 4.47	50.00 ± 4.47	58.00 ± 5.83	68.00 ± 3.74
Group 2	28.00 ± 2.00 0.0667	40.00 ± 3.16 1.0000	47.00 ± 2.00 0.5573	50.00 ± 3.16 1.0000	60.00 ± 3.16 0.7707	74.00 ± 4.00 0.3052
Group 3	22.00 ± 2.00 1.0000	34.00 ± 2.45 0.2731	44.00 ± 2.45 0.2731	50.00 ± 4.47 1.0000	60.00 ± 4.47 0.7924	68.00 ± 3.74 1.0000

***P-value is significant difference (<0.05)**

The result showed that there was no significant difference in the mean weight of animals in group 2 and 3 when compared to the control group.

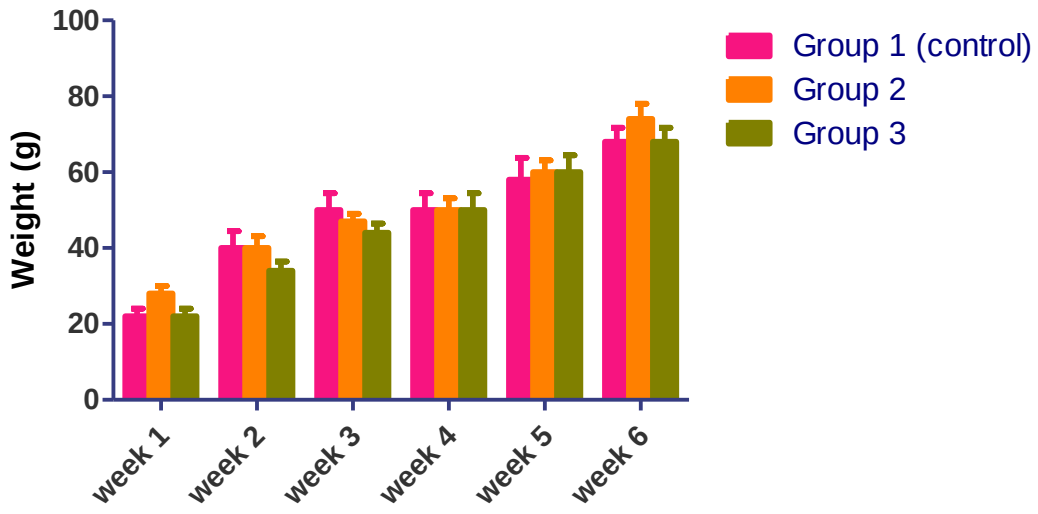

Figure 4: Graphical representation of the mean weight of the animals

DISCUSSION

This study was carried out to determine some of the effects of moringa oleifera on the microarchitecture of the cerebrum of developing wistar rats, average weight of the animals and also on the relative brain weight of the animals. Histological findings revealed that the control group (PLATE A) notes normal evenly distributed pyramidal neurons, normal perivascular space and glial cells. All these are in conformity with the normal histology of the cerebrum. The photomicrograph of group 2 (PLATE B) which received 100 mg/kg of the extract showed normal evenly distributed pyramidal neurons, enlarged perivascular space and scanty glial cells. This shows a slight distortion in the microarchitecture of the prefrontal cortex. The photomicrograph of group 3 (PLATE C) which received 200 mg/kg of the extract showed scanty pyramidal neurons, pyknotic pyramidal neurons and glial cells appears normal. This also shows a slight distortion in the microarchitecture of the prefrontal cortex. The results showed that feed intake of the animals in group 2 was significantly different compared to the control group from the 4th week of administration, while group 3 animals feed intake was significantly different from control throughout the six weeks of administration. This implies that moringa oleifera increases feed intake and also that its ability to do so is mainly dependent on the dose administered. Also, result showed that there was no significant difference in the mean weight of animals in group 2 and 3 when compared to the control group. There was also no significant difference in the relative brain weight of animals in group 2 and 3 compared to the control. The results obtained from this study showed that moringa oleifera increases feed intake , which is one of the multi-functions of the plant and also can be said to have a neuroprotective effect which might be due to the important constituents of moringa which includes proteins, vitamins, minerals, beta carotene, amino acids and various phenolics (Anwar, 2007).

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