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Noxious effect of *Moringa oleifera* leave extract on the developing brain, morphology and behaviour of Wistar rat

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Abstract

The effects of *Moringa oleifera* leave extracts on the morphology and behaviour of the *postnatal* developing rat brain was studied. Twenty pregnant rats of Wistar strain weighing between 160 g and 180 g were used in the study. The pregnant rats were divided into two groups of ten animals per group. Group I animals received distilled water and served as control animals, while group II animals received 200 mg/kg body weight of *Moringa oleifera* leave extract orally. All the animals were provided with rat cubes and water *ad libitum* during pregnancy and lactation. After birth, five pups of day 21 were weighed and subjected to behavioural study. Animals of days 1, 7, 14, 21 and 28 of age were sacrificed by cervical dislocation, the brain dissected out, weighed and fixed in 10% formol-saline for microscopic studies. Some congenital malformations such as meromelia, phocomelia and amelia were observed in the *Moringa oleifera* group only. There was a significant increase in body weight of the *Moringa oleifera* animals on days 7, 14 and 21, in brain weight on days 1 and 7, and in cerebral weight on days 1, 7 and 21 ($p<0.05$). The behaviour of the rats was significantly worse in the *Moringa oleifera* group, especially in the area of motor function. Microscopically, there was significant reduction in the cerebral cortical thickness of *Moringa oleifera* group on days 21 and 28. Immunohistochemical studies revealed poor myelination in the *Moringa oleifera* group. The results indicate that maternal consumption of *Moringa oleifera* significantly affected the general morphology as well as behaviour of their pups, therefore it should be consumed with caution in pregnancy until better knowledge on humans is available.

Key words

Cerebral cortex, plant extract, natural medicine, phytotherapy, embryology, development.

Introduction

The mammalian cerebral cortex is a complex structure that contains a diversity of neurons and has rich intrinsic and extrinsic connectivity. The cerebral cortex provides the biological basis for human cognitive capacity and is, arguably, the part of the brain that most distinguishes us from other species. Some neuroactive chemicals have been shown to induce long-lasting behavioural alterations upon exposure during critical periods, even at low doses and in the absence of acute neurotoxicity (Spyker, 1975). Accordingly, these chemicals have been classified as behavioural teratogens (Swaab and Mirmiran, 1985; Vorhees and Molinow, 1987).

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Moringa Oleifera (*M. oleifera*) Lam is a short, slender, deciduous, perennial tree of about 10 meter in height. *M. oleifera* is one of the best known and most widely distributed species of the monogeneric family *Moringaceae.* It is found wild and cultivated throughout the plains, especially in hedges and in house yards, thrives best under tropical insular climate, and is plentiful near the sandy beds of rivers and streams. It can grow well in the humid tropics or hot dry lands, can survive destitute soils, and is little affected by drought (Kumar et al., 2010) *M. oleifera* has many uses, some of which are as antihypertensive (Faizi et al., 1998), anti-inflammatory, antispasmodic and diuretic activity (Caceres et al., 1992), hypolipidemic (Ghasi et al., 2000; Mehta et al., 2003); it reduces arthritis, aids digestion (Verma et al., 1976) and combats malnutrition, since it has seven times the vitamin C content of oranges, four times the calcium content of milk, three times the potassium content of banana, four times the vitamin A content of carrot and two times the protein content of milk (Dahot, 1988). Almost all the parts of this plant - root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil - have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammatory and infectious diseases along with cardiovascular, gastrointestinal, haematological and hepatorenal disorders. The seeds of *Moringa* are considered to be antipyretic, acrid and bitter, and reported to show antimicrobial activity (Sastri, 1962). The seed can be consumed fresh as peas; or pounded, roasted, or pressed into sweet, non-desiccating oil, commercially known as 'Ben oil' of high quality.

However, research has shown that *M. oleifera* leave extract contains important phytochemicals (Kasolo et al., 2010, Kasolo et al., 2011) which are potential toxins to animals and insects depending on the amount ingested. Nath et al. (1992) reported that *M. oleifera* leaves is one of the plants used as abortifacient in India. This research investigated the effects of methanolic extract of *M. oleifera* on developing rat brain.

Materials and methods

Plant material

Fresh leaves of *M. oleifera* were plucked from a compound at Oluyole, Ibadan, Oyo State, Nigeria. The leaves of the *M. oleifera* were identified and authenticated at the Federal Research Institute of Nigeria (FRIN) with FHI No 109606. Phytochemistry was done in the Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria.

Preparation of *Moringa oleifera*

The *M. oleifera* leaves were rinsed in distilled water to remove possible dirt and fungi. The leaves were dried at room temperature and shredded into smaller pieces. Cold methanolic extract was concentrated with rotary evaporator and room dried. The *M. oleifera* extract was weighed and kept in the refrigerator. The extract was reconstituted with distilled water and the pregnant rats administered 200 mg/kg body weight orally.

Animals

Ten adult female Wistar rats weighing between 160 and 180 g were housed in the central animal house, Faculty of Basic Medical Science, University of Ibadan. Ethical approval for the use of laboratory animals was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) with approval No. UI-ACUREC/App/2015/012. They were randomly assigned into two groups; Group I served as control, Group II was fed with *M. oleifera* extracts. The animals were fed with rat cubes purchased from Ladokun Feeds, Ibadan, Oyo State and water provided *ad libitum*.

The animals were divided into two groups:

Group I: received distilled water orally and served as the control group.

Group II: received per os 200 mg (of *M. oleifera* leave extract)/kg body weight of rat.

At day 21 (D21) each pup was subjected to behavioural tests. The pups on D1, 7, 14, 21 and 28 were weighed, sacrificed by cervical dislocation, and the brain dissected out, weighed and fixed in 10% formol-saline for histology and immunohistochemistry.

Analyses and tests

The following analyses and tests were performed.

Phytochemistry: The extract of M. oleifera was screened for the following phytochemicals: alkaloids, cardenolides, anthraquinones, saponins, tannins and flavonoids.

Gross morphometry of rats: Body weight and brain and cerebral weights were measured using a digital weighing balance OHAUS AP250D (Mettler, Toledo, OH).

Behavioural tests: The following tests were done. **Surface righting test**, *i.e.* the number of seconds it took the animal to turn on its four limbs, was administered on day 1 and day 7 *postpartum* to explore behaviour; **negative geotaxis**, *i.e.* the time (seconds) it took the animal to re-orientate against gravity, was tested on day 21 to explore muscle function and coordination; **forelimb grip and open field motor** activities were tested on day 21 to explore motor function and locomotor activities respectively.

Histology: Brain slides were prepared by fixing in 10% formalin, embedding in paraffin and sectioning at 5 μm in an automated microtome. The sections were mounted on slides and stained with haematoxylin and eosin. The tissue was examined for inflammation and changes in cortical thickness.

Morphometry: The haematoxylin and eosin stained slides were used to determine the cortical thickness of the brain and the distance between the molecular layer and corpus callosum.

Immunohistochemistry: To demonstrate the extent of myelination in pups on D28, employing the method of Todorich et al. (2011) was employed. Briefly, deparaffinized sections were incubated in anti-mouse monoclonal myelin basic protein antibody (AbCam, UK) at a dilution of 1:500, overnight, and temperature of 4^{o}C, followed by secondary biotinylated anti-mouse antibody at a dilution of 1:200 for 1 hour. The secondary antibody was tagged by avidin-linked horse radish peroxidase and revealed with diaminobenzidine and hydrogen peroxide. Images were acquired with a Digital Microscope YJ-2005DN BIO-MICROSCOPE® (Ningbo Yujie Optical Instruments, Zhejiang, China), using TSView CxImage® Software, version 6.2.4.3 (Tucsen, Fujian, China).

Statistical analysis

The data obtained from the experiments were subjected to statistical analysis with students'*t*-test using SPSS v11.0 (SPSS Inc, Chicago, IL). The level of significance was set at $p<0.05$. Data are given as mean ± standard error of mean (SEM).

Results

Phytochemistry: Phytochemical screening of the leaves of *M. oleifera* showed that it contained alkaloids, cardenolides, saponins and tannins (Table 1).

Gross morphology: One of the mothers from *M. oleifera* group gave birth to five pups with three pups showing meromelia, phocomelia, amelia and absence of tail (Figure 1).

Body, brain and cerebral weight: In *M. oleifera* group, as compared with its respective control, there were significantly heavier body (days 7, 14 and 21), brain (days 1 and 7) and cerebrum (days 1, 7, and 21) weights (Table 2).

Behavioural tests: In the treated group, as compared with the control group, the surface righting time was significantly higher on day 1 (Table 3), the time to re-orientate against gravity was significantly higher on day 21, the fore limb grip time was significantly lower on day 21 and the time for line crossing, rearing, and grooming in open field was significantly higher on day 21 (Table 4).

Histology: By morphometric analysis, the cerebral cortex resulted significantly thicker on days 7 and 14, but thinner on days 21 and 28 in the

Table 1 – Phytochemical screening of the leaves of *M. oleifera*.

Phytochemicals	*Moringa oleifera*
Alkaloid	++
Cardenolides	++
Anthraquinones test	- -
Saponins	+
Tannins	+
Flavonoids	- -

+ = present in mild quantity, ++ = present in moderate quantity, -- = absent

Figure 1 – Day 1 rats from a mother treated with *M. oleifera* presenting some congenital malformations including, meromelia, phocomelia and amelia with absence of tail (arrows).

Table 2 – Mean body, brain and cerebral weight of animals on days 1, 7, 14, 21, and 28.

Groups	Day 1	Day 7	Day 14	Day 21	Day 28
Body weight					
Control	5.00±0.10	6.04±0.08	16.10±0.07	24.20±1.19	34.64±1.44
M. oleifera	5.62±0.10	8.88±0.42[a]	22.10±0.18[a]	30.30±1.17[a]	41.86±0.94
Brain weight					
Control	0.39 ±0.01	0.44±0.02	0.92±0.12	1.28±0.04	1.30±0.04
M. oleifera	0.51±0.02 [a]	0.60±0.01[a]	1.04±0.02	1.30±0.04	1.30±0.04
Cerebral weight					
Control	0.28 ±0.01	0.34±0.02	0.78±0.04	0.92±0.02	0.99±0.03
M. oleifera	0.35±0.02[a]	0.53±0.02[a]	0.84±0.02	1.06±0.04[a]	1.04±0.02

Values are given as mean±SEM (n=5). Mean body, brain and cerebral weight of animals is expressed in grams. [a]*p<0.05* compared with the control animals.

Table 3 – Mean and standard error of mean of surface righting test (SRT) on days 1 and 7.

Groups	SRTday1 (secs)	SRTday7 (secs)
Control	2.00±0.55	1.60±0.40
M. oleifera	3.50±0.43[a]	1.20±0.20

Values are given as mean±SEM (n=5), Mean surface righting of animals is expressed in seconds. [a]*p<0.05* compared with the control animals.

Table 4 – Mean and standard error of mean of motor function and open field tests on day 21 *postpartum*.

Groups	NG (secs)	FHG (secs)	Line Crossing (count)	Rearing (count)	Grooming (count)
Control	1.40±0.24	113±6.34	18.33±1.31	3.20±1.32	3.20±0.96
M. oleifera	2.20±0.49[a]	77±14.85[a]	45.00±3.00[a]	6.00±1.41[a]	4.75±0.65[a]

Values are given as mean±SEM (n=5). NG= Negative geotaxis; FHG= Fore hand grip. Mean time for motor function and open field count are expressed in seconds. [a]*p<0.05* compared with the control animals.

M. oleifera treated group as compared with controls (Figures 2 and 3, Table 5). Using myelin basic protein immunohistochemistry the control animals on day 28 showed strong myelin expression in the dorsolateral part of the corpus callosum, while *M. oleifera* animals on day 28 showed a relatively normal expression of myelin at the horn of the corpus callosum, but diffuse pallor towards the lateral part (Figure 4).

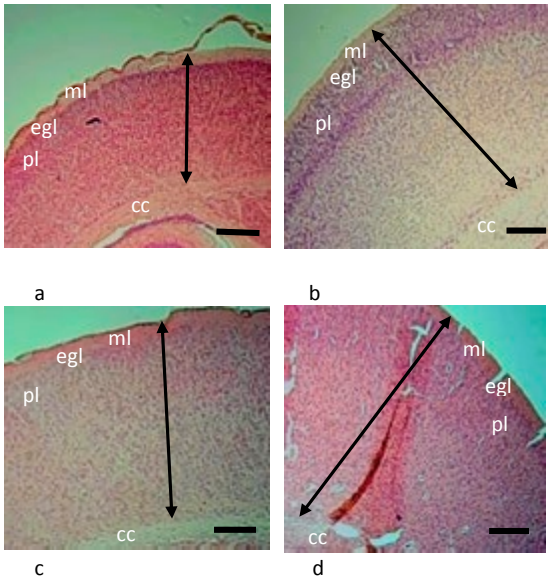


Figure 2 – Photomicrographs of the cerebral cortex of: a) control animal at day 7; b) *M. oleifera*-treated animal at day 7; c) control animal at day 14; and d) *M. oleifera*-treated animal at day 14, with uniform arrangement of cells (arrow showing increase in cortical thickness). Molecular layer (ML), External granular layer (EGL), Pyramidal layer (PL), Corpus callosum (CC). Haematoxylin and eosin. Scale: 100µm.

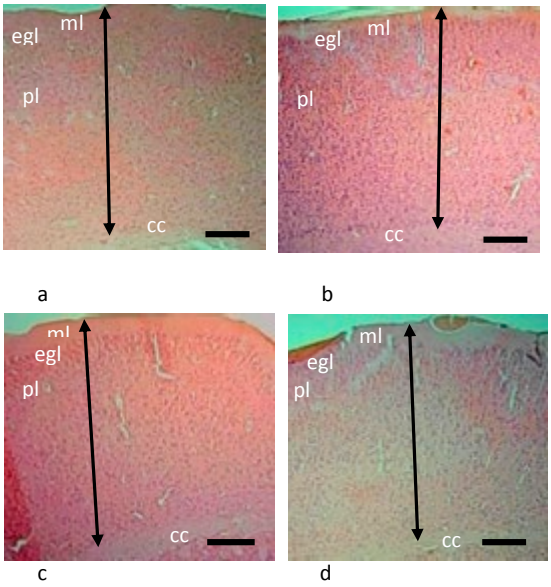
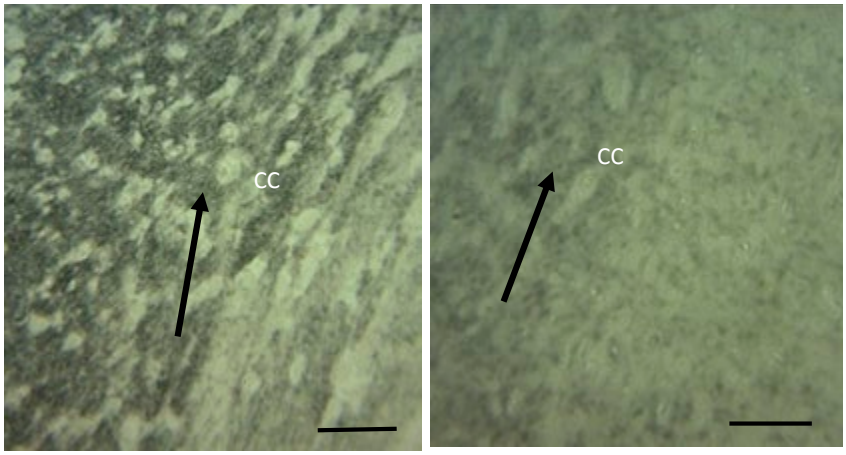


Figure 3 – Photomicrographs of the cerebral cortex of: a) control animal at day 21; b) *M. oleifera*-treated animal at day 21; c) control animal at day 28; and d) *M. oleifera*-treated animal at day 28, with uniform arrangement of cells (arrow showing increase in cortical thickness). Molecular layer (ML), External granular layer (EGL), Pyramidal layer (PL), Corpus callosum (CC). Haematoxylin and Eosin. Scale: 100µm.

Table 5 – Cerebral cortex thickness of rats on days 1, 7, 14, 21and 28

Group	Day 1	Day 7	Day 14	Day 21	Day 28
Control	0.43±0.02	0.55±0.01	1.00±0.01	1.29±0.01	1.74±0.02
M. oleifera	0.42±0.01	1.07±0.01[a]	1.37±0.01[a]	1.21±0.00[a]	1.53±0.00 [a]

Values are given as mean±SEM (n=5). Mean cerebral cortical thickness is expressed in mm. [a]*p<0.05* compared with the control animals.



a b

Figure 4 – Photomicrographs of corpus callosum (CC) of: a) Control animal day 28 showing strong myelination (arrow), b) M. oleifera day 28 showing mild myelination (arrow). Immunostaining for myelin basic protein. Scale: 20µm

Discussion

Morphological defects of the limbs (amelia, meromelia, phocomelia) were seen in three one-day-old pups from one *M. oleifera*-treated female rats. There was significant body, brain and cerebral weight gain in the *M. oleifera* treated animals. Increased body weight was reported by Ghebreselassie et al. (2011) following administration of *M. oleifera* extract in rats. The reason for this increased weight gain found in the *M. oleifera*-treated rats was not completely understood, however, Ikegwuonu et al., (2010) reported that oedema and inflammation as a result of brain damage could bring about increased brain mass. In the present study there was a co-existence of poor myelination in the corpus callosum and decreased cortical thickness in post weaned *M. oleifera*-treated rats; the cortical thickness of the cerebrum has been considered a very important morphological trait in many brain studies (Lee et al., 2011). Therefore, the reduction in the cortical thickness may be hypothesized to have been caused by *M. oleifera* leave extract.

This might have led to a significant reduction in motor functions as observed in the fore limb grip and open field tests. In the *M. oleifera* group there was a signifi-

cant decrease in the motor function as tested by surface righting test on day 1 old rats, when compared to the control. Negative geotaxis and fore limb grip tests also followed this same trend. This may suggest that *M. oleifera* caused an insult to the sensorimotor cortex, leading to muscular weakness (indicated in the *M. oleifera* treated group by decreased time spent in the fore hand grip test), reduced muscle coordination and orientation and impaired sensory feedback to perform muscle movement. This would be in line with the observations of Alvarez and Emory (2006). Hyperactivity was observed in the *M. oleifera* through the open field test, especially by the number of grooming episodes, this is in accordance with the report of Umoren et al., (2009).

In conclusion, consumption of *M. oleifera* could affect the body, brain and cerebral weight and cause poor myelination of the white matter, reduced cortical thickness and neurobehavioural defects. This study suggests that *M. oleifera* may be embryotoxic as well as teratogenic in animals. Further studies should be done to ascertain the level of *M. oleifera* embryotoxicity and teratogenicity.

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