Asian Journal of Advances in Medical Science

3(4): 222-231, 2021

EFFICACY OF UNRIPE *Musa sapientum* WHOLE FRUIT ON NEUROMUSCULAR STRENGTH AND OLFACTORY RESPONSE LINKED TO CEREBRO-CEREBELLAR IONIZED CALCIUM, MUSCLE CREATINE KINASE AND PLASMA ACETYLCHOLINESTERASE

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Received: 27 August 2021
Accepted: 03 November 2021
Published: 09 November 2021

Original Research Article

ABSTRACT

The performance of daily activities is dependent on several factors, such as strength, balance, flexibility, endurance and perception of sensations of special senses. Unripe *Musa sapientum* is one of the most cultivated tropical fruit in the world, with scarce literature concerning its neurologic relevance. This study determined the tendency for Unripe *Musa sapientum* whole fruit hydromethanolic extract to influence neuromuscular strength, endurance and olfaction in normal condition. Peak absorbance was read at 200 to 1100 nm using ultraviolet visible spectrophotometry to determine some bioactive functional groups in unripe *Musa sapientum* whole fruit extract. The test groups *ii-iv* were orally subjected to graded doses of unripe *Musa sapientum* whole fruit; 100, 150 and 200 mg/kg respectively, all compared to control group *i*. There was a significant dose-dependent improvement in olfactory response, neuromuscular strength and endurance. Unripe *Musa sapientum* extract caused a dose-dependent significant decrease in plasma creatine kinase but increased acetylcholinesterase and ionized calcium in cerebral cortex and cerebellum. The effectiveness of the extract on the tested parameters was progressive and its efficacy may be dose-dependent.

Keywords: Muscle; olfaction; performance; *M. sapientum*; calcium.

ABBREVIATIONS

UR = *Unripe*
UV-vis = *Ultraviolet visible spectrophotometry*
CK-MM = *Muscle isoform creatine kinase*
AchE = *Acetylcholinesterase*

1. INTRODUCTION

Voluntary body movements involve physical, biochemical and psychological changes [1,2]. Several research scientists have investigated the role of the central nervous system in body movements from conscious to subconscious level [2]. Ancient Greek

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philosophers, most especially, Alcmaeon, Herophilus, Praxagoras and Erasistratus, contributed to the discovery of the human nervous system by dissecting human cadavers [1]. Motor nerves are connected to skeletal muscles, while sensory nerves connect with organs, and are responsible for perception of sensations [3,4]. The motor areas of cerebral cortex initiate voluntary movements and together with the cerebellum coordinate movement producing a fine, skillful and smooth output [5]. Some health benefits of physical activity and exercise, both characterized by an underlying neuromuscular activity, include favorable physiological, psychological and biochemical changes [1]. The sense of olfaction is the dominant sensory perception for many animals [5,6]. The sense of smell is an essential chemical signaling system that regulates food ingestion and interpersonal relations [6]. Disorders of olfactory system may cause difficulty in appreciation or recognition of odoriferous substances [7]. Other adverse effects of olfactory abnormalities may include weight loss, anxiety, personal hygiene uncertainty, mood swings and depression, feelings of vulnerability, regression of physical activities, social interactions and sexual life [6,7]. *Musa sapientum* (banana) is one of the most cultivated tropical fruit in the world [8]. Different parts of the banana plant contain carotenoids, phenolic compounds, and biogenic amines like dopamine, serotonin, noradrenaline, tryptophan, and tyrosine, which are relevant to the pathophysiology of mental disorders [9]. Studies in experimental animal models have confirmed the therapeutic potential of the extracts obtained from different parts of this plant and these extracts have been shown to possess antioxidant activities [9,10]. The antioxidant activity of *M. sapientum* extracts reported by previous studies may be implicated in its potential neurologic manifestations [11]. The present study was undertaken to investigate the dose-dependent effect of unripe *Musa sapientum* whole fruit extract on neuromuscular strength, endurance and olfaction.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh unripe *M. sapientum* whole fruit was collected from a plantation in Ifite-Nteje, Anambra state. The plants were not exposed to any form of preservative or ripening agent prior to and after selection. All samples were collected from same bunch with white pulp and green peel physical appearance.

2.2 Plant Identification

The plant was identified in the Herbarium of Department of Plant Science and Biotechnology, University of Port Harcourt with reference UPH/PSB/2020/014.

2.3 Extract Preparation

The extraction solvent used was water and methanol in a ratio *1:4*. The dried sample weight was 735 grams while solvent volume was 10 liters.

2.4 Toxicity Study

Using Locke's method, the Oral lethal dose 50 (LD_{50}) of Unripe *M. sapientum* whole fruit is >5000mg/kg. The extract is relatively non-toxic.

2.5 Ultraviolet Visible Spectrophotometric Analysis

UV-visible spectrophotometric analysis was conducted on the sample using a UV-visible spectrophotometer (Apel 3000UV) with a slit width of 2nm, using a 10-mm cell at room temperature. The unripe whole fruit extract was examined under visible and UV light in the wavelength ranging from 200-1100nm. This facilitated understanding of the various peaks arising due to multiple components present in the extract as well as to find out the wavelengths at which maximum absorbance was observed.

2.6 Study Design

This study included a control group (*i*) and three (3) separate test groups (*ii*, *iii* and *iv*) given graded doses of unripe *M. sapientum* whole fruit extract. Only male wistar rats weighing 160 to 180grams were sampled. The study duration was 56 days with samples taken in regular intervals.

Table 1. Treatment protocols

Groups	Protocols
i	Feed and water
ii	UR *M. sapientum* whole fruit 100mg/kg
iii	UR *M. sapientum* whole fruit 150mg/kg
iv	UR *M. sapientum* whole fruit 200mg/kg
N=6	

The animals were fed standard food pellet and water *ad libitum* and exposed to regular 24 hours day/night rhythm. Proper care was ensured to avoid any form of stress, pain or restraint.

2.7 Neurologic Tests

2.7.1 Test for olfactory response

2.7.1.1 Buried feed test (BFT)

The animals were fasted overnight before been introduced into clean cages 46cm x 23.5cm x 20cm. The rewarding (food) stimulus was buried in 3cm fresh cage bedding. Kellogg's® cookies was used for this test. Each animal was allowed to acclimate to the cage for 5 minutes after which the latency period (in seconds) taken to find the food stimulus was carefully recorded. This test was conducted before and after the experimental period. Latency of olfaction using buried food test was assayed twice; before and after treatment period.

2.7.2 Test for neuromuscular strength

2.7.2.1 Horizontal grid test (HGT)

A grid was placed horizontally, with the animals carefully place on it ensuring tight grip with both fore and hind limbs. The grid was turned upside down and suspended 18" from surface. All aversive stimuli were removed from test area. The duration of time (in seconds) the animal cling to the grid before falling completely off the grid was recorded.

2.8 Tissue Sampling

At the end of the eight weeks treatment period, rats were killed by cervical dislocation and the fore and hind brains (including the cerebral areas and cerebellum) were isolated on ice and then divided into 2 sagittal sections using a sharp blade. One part was stored in ice-cold sterile physiologic saline at -20 °C. They were fixed in 10 % phosphate-buffered formalin for 48 h for histological examination.

2.9 Brain Tissue Homogenization

This was done using standard procedures by Ilochi, et al., [12]. The brain tissue was cut into thin slices of appropriate sizes for analysis (100 to 300mg) and placed into a microcentrifuge tube. The typical sample size was 100mg. The tissue was washed properly with 1 ml PBS. Glass beads (0.5mm) equal to tissue mass followed by 0.1 to 0.6ml of buffer was added. The micro-centrifuge tubes were placed into blender at speed 6 at time 3 to homogenize.

2.10 Biochemical Tests

2.10.1 Brain ionized calcium assay

Calcium is measured using a Potentiometer. This method determines the potential difference that develops between the inner and outer phases of an ion selective electrode. The electrode is made of a selectively permeable material to calcium ion. The potential is measured by comparing it to the potential of reference electrode. Since the reference electrode has a constant potential, the voltage difference between the two electrodes is attributed to the concentration of ionized calcium in the sample.

2.10.2 Plasma acteylcholinesterase detection

Acetylcholinesterase (AChE) activity in the different brain regions was determined by Ellman's method, using acetylcholine iodide as a substrate. In this method AChE in samples hydrolyzes acetylthiocholine iodide into thiocholine and butyric acid. The thiocholine reacts with 5, 5′-dithiobis-2-nitrobenzoic acid to form 5- thio-2-nitrobenzoic acid. Using a spectrophotometer, the yellow color developed is measured at 412 nm.

2.10.3 Plasma Creatine kinase detection

This was measured using Creatine kinase assay kit; BioAssay Systems EnzymChrom™ Creatine Kinase Kit (ECPK-100) at -20^{o}C storage temperature and detection range of 5 to 300U/L in 96-well plate assay. The principle of this reaction was based on enzyme-coupled reactions and absorbance change measured at 340nm proportionate to creatine kinase activity in the sample.

2.11 Tissue Analysis

2.11.1 Histological examination

Fixed brain specimens were processed and embedded in paraffin. Serial four μm-thick sections were made, stained with haematoxylin and eosin (H&E) and examined by light microscopy.

2.12 Statistical Analysis

In other to properly translate the outcome of this study, data collected from this study was analyzed using One-Way analysis of variance (ANOVA) and Post Hoc analysis with the aid of IBM®SPSS Version 20.0. The Percentage change (*%c*) was also presented in standard charts and tables, adopting methods used by Chuemere, et al. [13].

3. RESULTS

The following results were obtained from this study;

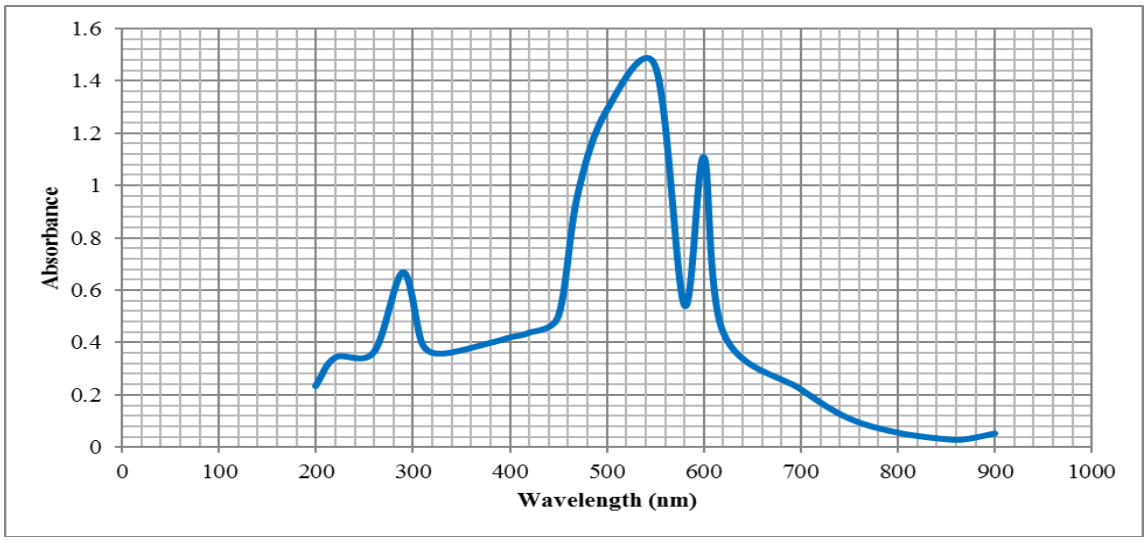

Fig. 1. UV visible spectrophotometric representation of the various peak absorbances in unripe *M. sapientum* whole fruit hydromethanolic extract

From Fig. 1, absorbance was read from 200 to 1100 nm. Peak absorbance was read at the wavelengths 220, 290, 550, 600 and 900 nm.

From Table 2, there was a significant decrease in latency in treatment doses 100, 150 and 200 mg/kg compared to control. This significant change in olfactory response was higher on day 54 for same treatments. The unripe *M. sapientum* extract, through latency and when compared to the control, showed it can improve olfactory response.

Table 2. Olfactory response to graded unripe *M. sapientum* whole fruit treatments

Groups	**Latency (600 sec. max)**					
	0	***%c****	**27**	***%c****	**54**	***%c****
Control	385.8±0.80	-	385.5±1.50	-	386.0±1.40	-
UR *M.s.* Whole fruit (100 mg/kg)	386.0±1.20	0.05	334.6±5.23*	-13.2	290.2±8.43*	-24.8
UR *M.s.* Whole fruit (150 mg/kg)	384.0±0.90	-0.47	296.2±6.11*	-23.2	262.3±5.96*	-32
UR *M.s.* Whole fruit (200 mg/kg)	384.3±0.90	-0.39	283.9±1.70*	-26.4	247.3±4.10*	-35.9
Total	**1540.1**		**1300.2**		**1185.8**	
Average	**385.03**		**325.10**		**296.50**	

Table 3. Regular time interval-dependent olfactory response to graded unripe *M. sapientum* whole fruit treatments

Groups	**Latency (600 sec. max)**					
	Day 0	***%c*** $^{0-27}$	**Day 27**	***%c*** $^{27-54}$	**Day 54**	***%c*** $^{0-54}$
Control	385.8±0.80	-0.07	385.5±1.50	0.13	386.0±1.40	0.06
UR *M.s.* Whole fruit (100 mg/kg)	386.0±1.20	-13.3	334.6 ± 5.23^{0}	-13.2	$290.2\pm8.43^{0,27}$	-24.83
UR *M.s.* Whole fruit (150 mg/kg)	384.0±0.90	-22.8	296.2 ± 6.11^{0}	-11.4	$262.3\pm5.96^{0,27}$	-31.6
UR *M.s.* Whole fruit (200 mg/kg)	384.3±0.90	-26.0	283.9 ± 1.70^{0}	-12.8	$247.3\pm4.10^{0,27}$	-35.6
Total	**1540.1**		**1300.2**		**1185.8**	
Average	**385.03**		**325.10**		**296.50**	

From Table 3, unripe *M. sapientum* extract, from day 27, time-dependently reduced latency. Percentage change further revealed the improvement in olfactory response marked by a higher reduction in latency was observed accordingly unripe *M. sapientum* whole fruit extract 200>150>100 mg/kg.

From Table 4, there was no significant difference in ionized calcium present in cerebral cortex except at 200 mg/kg unripe *M. sapientum* whole fruit extract treatment. The percentage change showed clear progression in all treatment values relative to control.

From Table 5, there was a significant increase in ionized calcium ion present in cerebellum compared to control for all 3 doses of *M. sapientum* whole fruit extract treatments with slight difference in percentage change between 100 mg/kg and 150 mg/kg extract treatments.

From Table 6, there was a negative correlation between the dose of unripe *M. sapientum* extract treatment and creatine kinase but a positive correlation compared to acetylcholinesterase. The *M. sapientum* whole fruit extract at the studied dose showed reverse effect on both muscle function markers.

Table 4. Changes in ionized calcium in cerebral cortex in response to graded unripe *M. sapientum* whole fruit treatments

Groups	Ca (mmol/L)	*%c**
Control	2.12±0.90	-
UR *M. s.* Whole fruit(100 mg/kg)	2.22±0.70	4.7
UR *M. s.* Whole fruit (150 mg/kg)	2.37±0.80	11.8
UR *M. s.* Whole fruit (200 mg/kg)	2.48±0.77*	16.9
Total	**9.20**	
Average	**2.30**	

Table 5. Changes in ionized calcium in cerebellum in response to graded unripe *M. sapientum* whole fruit treatments

Groups	Ca (mmol/L)	*%c**
Control	2.16±0.11	-
UR *M. s.* Whole fruit (100 mg/kg)	2.40±0.63*	11.1
UR *M. s.* Whole fruit (150 mg/kg)	2.41±1.54*	11.6
UR *M. s.* Whole fruit (200 mg/kg)	2.48±1.73*	14.8
Total	**9.45**	
Average	**2.40**	

Table 6. Muscle function changes in response to graded unripe *M. sapientum* whole fruit treatments

Groups	CK-MM (U/L)	%c*	AchE (U/Ml)	%c*
Control	57.27±0.44	-	36.32±0.57	-
UR *M. s.* Whole fruit (100 mg/kg)	40.80±0.77*	-28.8	43.82±0.25*	20.6
UR *M. s.* Whole fruit (150 mg/kg)	38.00±0.21*	-33.6	45.27±0.46*	24.6
UR *M. s.* Whole fruit (200 mg/kg)	36.37±0.13*	-36.5	49.42±0.38*	36.1
Total	**167.44**		**174.83**	
Average	**41.90**		**43.71**	

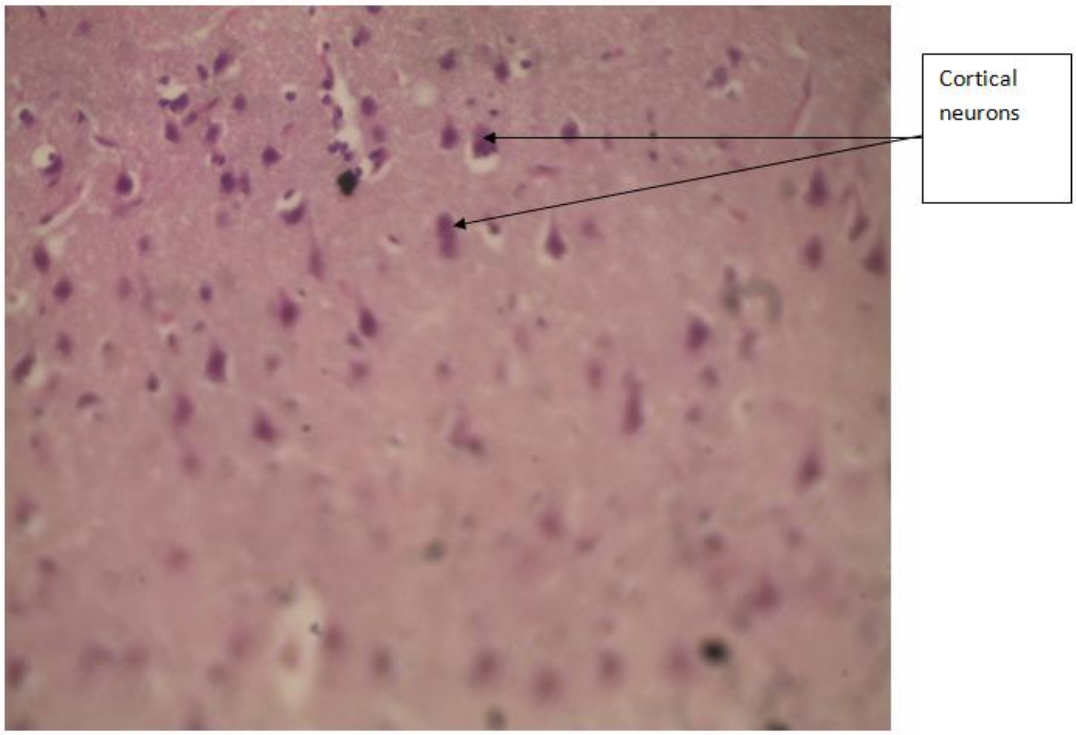

Plate 1. Photomicrograph Representation of control cerebral cortex (Mag.X600) shows the large pyramidal cells in cerebral cortex

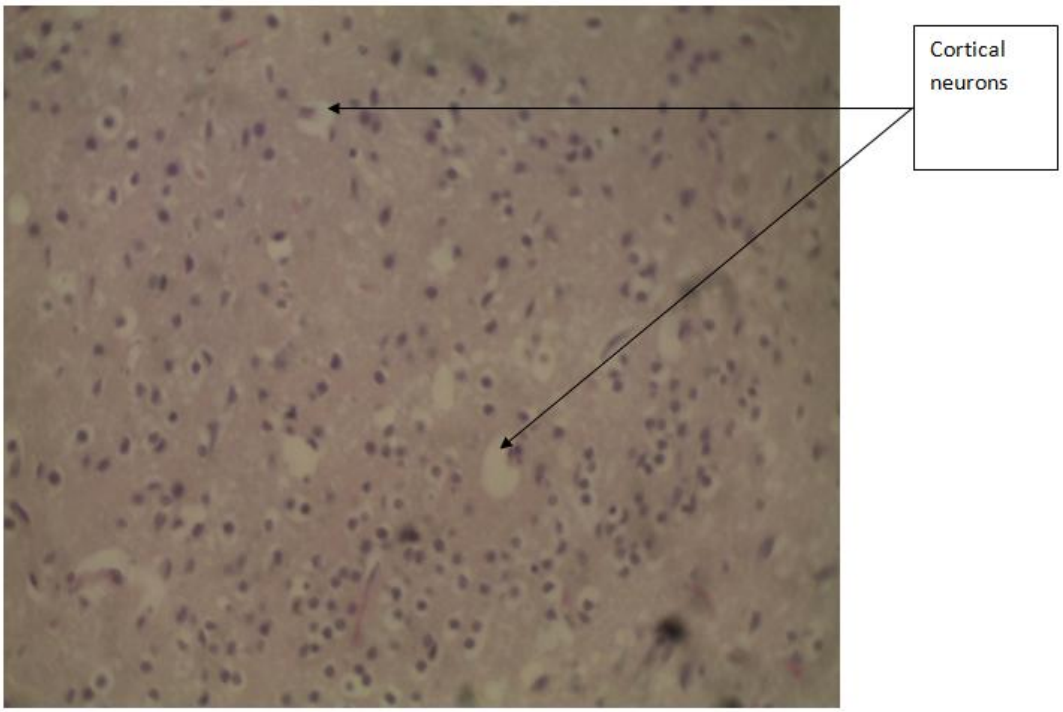

Plate 2. Photomicrograph Representation of Cerebral cortex of unripe *M. sapientum* whole fruit 200 mg/kg (Mag.X600) show large pyramidal cells in cerebral cortex similar to control

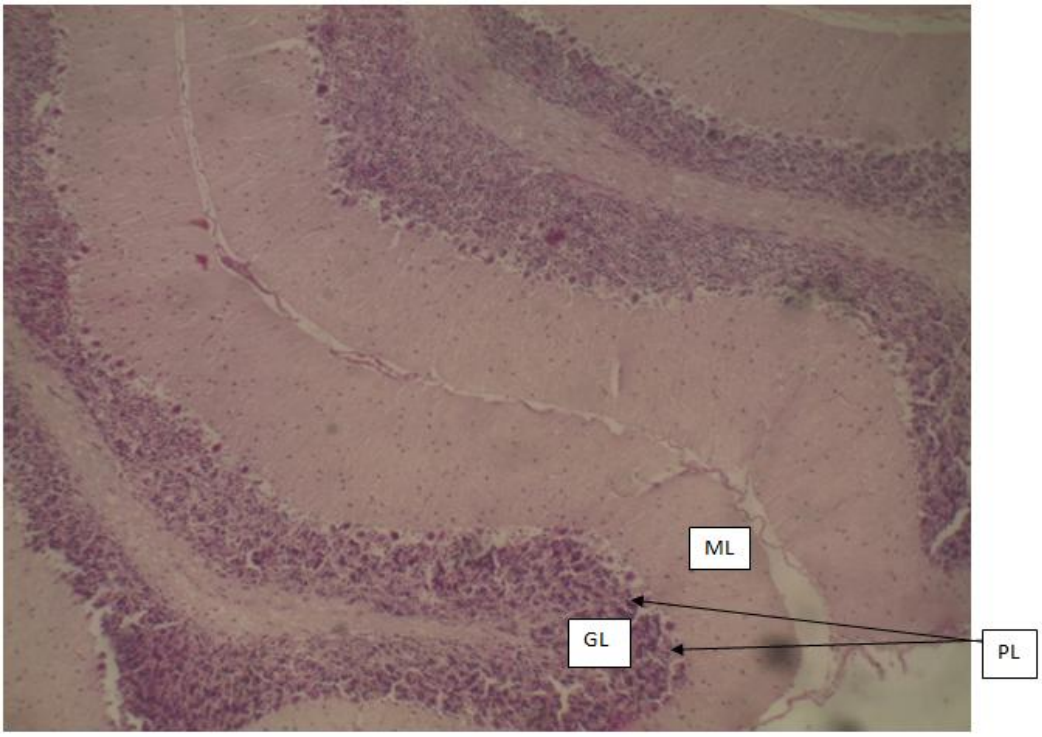

Plate 3. Photomicrograph representation of cerebellum of control (Mag.X125), the cerebellar cortex shows three (3) distinct layers of cells: purkinje layer (PL), molecular (ML) and granular layer (GL)

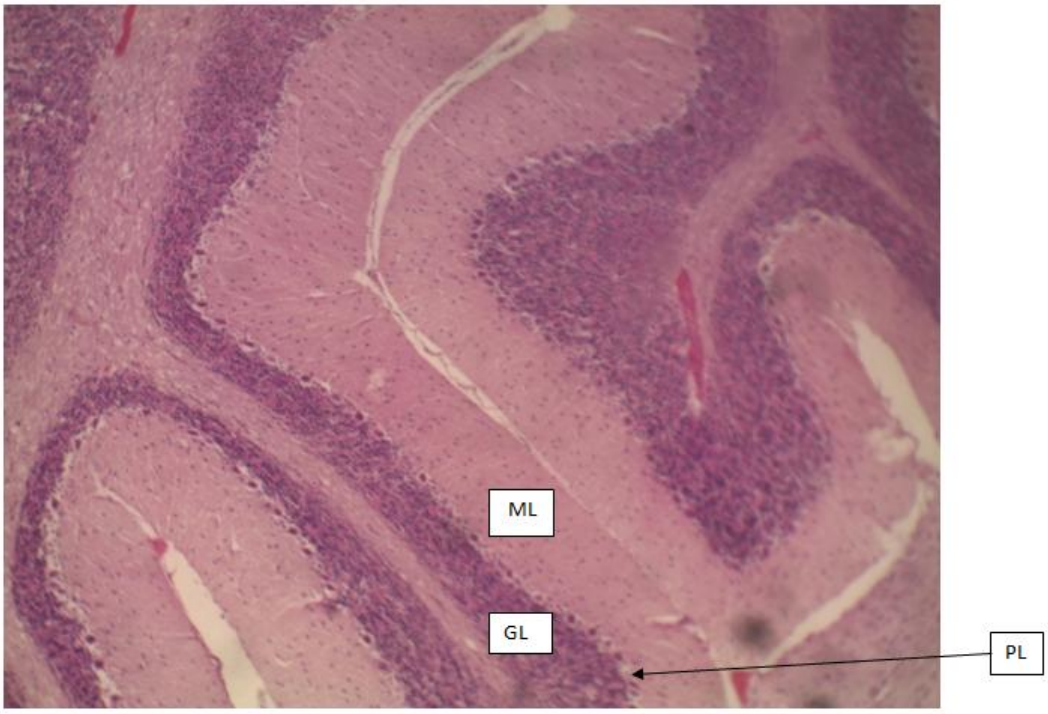

Plate 4. Photomicrograph representation of cerebellum of unripe *M. sapientum* whole fruit 200 mg/kg (Mag.X125), cerebellar cortex shows three (3) distinctive cell layers: purkinje layer (PL), molecular (ML), and granular layer (GL) as in the control

4. DISCUSSION

This study aimed at determining the neurologic tendency of unripe *M. sapientum* whole fruit hydromethanolic extract on neuromuscular strength, endurance and olfaction in male wistar rats. UV visible spectrophotometric representation of the various peak absorbances in unripe *M. sapientum* whole fruit hydromethanolic extract showed it is composed of several functional or bioactive chemical groups which include anthocyanins, rutin, catechins as flavonoids and ribalinindine as quinoline alkaloids which is in correspondence to earlier translational reports [14]. The extract at regular intervals was able to enhance olfactory response. The dose of the extract administered correlated positively with the latency and same was observed in neuromuscular strength and endurance. *M. sapientum* extract may have the ability to influence both the nervous and musculoskeletal systems and the mechanistic link for its action may be targeted at the central level of control; the central nervous system. Composed of several phenolics [14] [15] and as revealed in UV-Vis in this study, the extract or its bioactive constituents may have the spectacular character of crossing the phospholipid membrane bilayer of the blood brain barrier (BBB) [16]. Substances present in unripe *M. sapientum* whole fruit have the ability to cross the barrier due to the fact that they are lipid-soluble phenolic derivatives. Several metabolic intermediates are known to interfere with physiologically important signal transduction cascades even in normal conditions [16,17]. The removal or 'mop up' of these intermediates may be the likely cause of any beneficial result as seen after the extract treatments. The dose was able to affect the responses and outcomes possibly as a result of the increased bioavailability of the extract or its components within the CNS environment prolonging its duration and potentiating its actions. By increasing the level of ionized calcium, unripe *M. sapientum* whole fruit hydromethanolic extract confirmed a possible link connecting cerebral cortical and cerebellar ionized calcium concentration, olfactory signal transduction and neuromuscular transmission leading to end plate potential (EPP). The mechanism may be within the matrices of calcium-induced-calcium-release (CICR), whereby calcium signals within the motor areas of cerebral cortex influences calcium signals in corticocerebellum [18], i.e. the lateral areas of cerebellar hemispheres including lateral areas of lobulus ansiformis and lobulus paramedianus and the spinocerebellum [18,19,20]. Unripe *M. sapientum* whole fruit extract caused a decrease in plasma creatine kinase but an increase in plasma acetylcholinesterase. An increased plasma level of muscle-type creatine kinase (CK-MM) [18] assayed in this study as a muscle function marker is a clinical index of muscle necrosis or myofibril degeneration [19]. The reduction of creatine kinase enzyme in plasma shows the extract maintained the functional intergrity of the independent muscle fibers [18,21]. The contractile state of a particular muscle fiber may be adversely affected if the catalysis within it is defective. Acetylcholinesterase is a cholinesterase present in cholinergic synapses that metabolizes acetylcholine, an excitatory neurotransmitter, to acetate and choline. The activity of acetylcholinesterase is necessary for continuous transmission of signals across a synapse [22,23,24]. Unripe *M. sapientum* whole fruit extract may have increased the concentration of acetylcholinesterase in basal lamina of synaptic cleft thereby reducing its presence in plasma. The acetylcholinesterase detected in plasma was significantly reduced progressively as the dose of *M. sapientum* hydromethanolic extract was increased.

5. CONCLUSION

Unripe *M. sapientum*, commonly ingested as ripe or in pulp fruit form, may significantly be of neurologic benefit when ingested as whole fruit. Its bioactive components, probably through central calcium regulatory mechanisms may potentiate or enhance some neurobehavioral responses like olfaction and neuromuscular strength.

6. RECOMMENDATION

This study was executed within the matrices of neurosciences. The effect of the studied extract on other physiologic systems may be investigated to further expand its literature.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study followed the already existing principles and guidelines of animal use in research and was approved by University of Port Harcourt Research Ethics Committee. The approved standard of animal use was developed by the National Committee for Research Ethics in Science and Technology (NENT), 2018, and endorsed by the Norwegian National Research Ethics Committee 2016/63/EU.

ACKNOWLEDGEMENTS

We thank all staff and students of Department of Human Physiology, Madonna University and University of Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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