IAR Journal of Medical Sciences
ISSN Online: 2708-3594
Frequency : Monthly
Language : English
Origin : Kenya
Website : https://www.iarconsortium.org/journal-info/iarjms

OPEN ACCESS JOURNALS

Research Artticle

Cerebellar Cortex and the Behaviour of Mice (*Mus Musculus*) Following the Administration of Palm Wine

Article History

Received: 25.06.2020
Accepted: 22.07.2020
Revision: 07.08.2020
Published: 12.08.2020

Author Details

F.E Oladipupo[1], O.J Ogundipe[2], O.F. Akinpelu[1], S.O. Jimoh[1], O.G Ajao[3], and O.M Onasanya[1]

Authors Affiliations

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne Campus, Ogun State, Nigeria

[2]Department of physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile - Ife, Osun State, Nigeria

[3]Department of Anatomy, Faculty of Basic Medical Sciences,University of Ilorin, Kwara State, Nigeria

Corresponding Author*
Ogundipe Dare Joshua
Email: darejoshua97@gmail.com

How to Cite the Article:
E Oladipupo, O.J Ogundipe, O.F. Akinpelu, S.O. Jimoh, O.G Ajao, and O.M Onasanya(2020); Cerebellar Cortex and the Behaviour of Mice (*Mus Musculus)* Following the Administration of Palm Wine **.** *IAR J MedSci, 1*(3)155-168.

Abstract: ***Aim:***-To investigates the histomorphological studies of the cerebellar cortex and the behaviour of mice after palm wine administration. ***Material and Method:-*** 40 adult mice (25-30g) were grouped into (A, B, C and D) comprised of 10 mice each. They were kept in plastic cages and fed with feed and water *ad libitum*. Group A (control group) received distilled water, group B ,C and D received 14.3ml/kg daily palm wine,14.3ml/kg palm wine every other day and 14.3ml/kg 50% diluted palm wine every other day respectively, orally for four weeks. They were weighted using weighing balance, the neurobehavioral evaluation was performed using the open field maze and the health status was appraised by the rectal temperature. After the last dose of administration, the animals were anaesthetized; the cerebellum was excised, weighed and fixed in 10% formal saline for H&E, Cresyl fast violet and bieschowsky silver stain techniques. Data were analyzed using one way ANOVA at $p<0.05$ followed by a posthoc test, results were expressed as mean $\pm$ S.E.M. ***Results;-*** decrease in the rectal temperature of the entire treated group when compared to the control group, relative weight of the cerebellum was higher in the treated group compared to control, neurobehavioral observations (motor parameters) showed difference between treated mice compared to control. Histological observations showed vacuolation and slight degeneration of Purkinje neuron cell bodies except in group D animals. ***Conclusion:-*** The study showed that palm wine intake on regular basis may affect the histoarchitecture of the cerebellum, neurobehavioural activities such as locomotion and balancing.

Keywords: Neurobehavioral, Palm wine, Cerebella cortex, Vacuolation, Degeneration**.**

INTRODUCTION

The cerebellum is often referred to as the little brain or the neuronal machine. It is the hindbrain situated in the posterior cranial fossa. The cavity of fourth ventricle separates it from medulla oblongata and from the cerebrum by the tentorium cerebelli (Saab, C. Y., & Willis, W. D. 2003). It is located at the base of the brain with large mass of cerebral cortex superiorly; it is a part of the metencephalon and divided into two hemispheres which contain narrow midline zone called the vermis (Agbeniyi, S.O., & Ayodele, M.S. 2010). It is important in motor control and equilibrium of the body. The presence of large number tiny granular cells in cerebellum makes it contains more neurons than the rest of the brain and receives about 200 million input fibers (Bell C.C, Han, V., & Sawtell, N.B. 2008).

The cerebellum is the primary center of motor coordination in the central nervous system (CNS). Hamre *et al.*, (1998) reported that "cerebellum is divided into a central vermis, which is flanked by lateral hemispheres, the vermis and hemispheres are subdivided by a series of parallel fissures defining a conserved pattern of folia". Its function depends on the actions of three principal neuronal subclasses; (i) granular cells, (ii) Purkinje cells, and (iii) deep cerebellar neurons (Hamre, K., & Goldowitz, D. 1998).

PALM WINE

Palm wine is produced by the natural fermentation of the sap obtained from tropical plants of the *Palmae* family (Okafor, N. 1978). Palm-wine is consumed by over 10 million people in Africa (Noll, R.G. 2008). It is a whitish liquid produced by fermentation of the sap of *Elaeis guineensis* and *Raphia hookeri* palm trees (Agu, R. C. *et al.*, 1999). Sugars such as sucrose, glucose, fructose, cellobiose, maltose, xylose, arabinose and galacturonic acid are the component found in the sap. (Faparusi, S. I. 1981) *Raphia hookeri* (Raffia palm) is the largest palm in Africa and is restricted to the tropical rainforest (Ndon, B.A. 2003). Its leaves are used for shelter and the stem produces palm sap, which is drank as beverage. The fermented sap could be distilled into alcohol or local gin (Martinez, M. C. *et al.*, 1992). The trunk could serve as firewood and mesocarp of the ripe fruit yields edible palm oil (Otedoh, M. O. 1990).

Palm wine has been used locally to extract active ingredients from leaves, barks and stems of some medicinal trees that are used in the treatment of various ailments such as malaria, dental problems, yellow fever and skin rashes in children (Uraih, N., & Iwuagbe, Y. 1990). Palm wine has a strong cultural significance among Africans. It is used at important traditional ceremonies like marriages, worship rites and other festivals. Although other types of wine are available, palm wine is irreplaceable for its well known health benefits as well as its cultural significance among Africans, especially the people of West Africa (Okpatu. 2013). Palm wine is used for making vinegar as acetification occurs after alcoholic fermentation of the wine in Asia. It is consumed fresh by pregnant women for its sweetness and nutrition while nursing mothers drink it warm to enhance breast milk production (Chandrasekhar, K. *et al.*, 2012).

MATERIALS, INSTRUMENTS AND REAGENTS

Materials: Forty adult male Mice, cotton wool, specimen bottles, Pelletized feed, thermometer, methylated spirit, , hand gloves, open field maze, stop watch, video camera, Distilled water, filter papers and beaker,

Instruments: Dissecting kit, dissecting tray, Syringe, oral canula, sensitive weighing scale, stop watch and video camera

Reagents: 10% formol saline, Alcohol (ascending grade), Heamatoxylin and Eosin stain, Chloroform, Cresyl Fast Violet stain and Bielschowsky's Silver stain.

PROCUREMENT OF PALMWINE

Freshly tapped palm wine from Raphia palm tree (*Raphia hookeri*) was purchased from a local palm wine tapper in Aiyepe town, Ogun state, Nigeria.

Source of Palm Wine Samples Used And Filtration

The sap was collected from the cut flower of the palm tree. A bottle is fastened to the flower stump to collect the sap. The freshly tapped samples were collected using sterilized labeled 1Litre capacity sample bottles with screw caps. The method of collection according to Obire (2005) was adopted in order to reduce fermentation rate. The palm wine was filtered with the aid of a filter paper to remove the suspended particles before administration. The dosages were in ml/kg body weight. Palm wine was administered orally with the aid of orogastric tube.

Experimental Animals Procurement, Housing and Handling:

Forty male adult mice (*mus musculus*) (25 - 30g) were used for all the investigations. All animals were maintained on suitable nutritional and environmental conditions throughout the experiment. They were fed with growers' marsh pellet diet (obtained from Top Feeds Limited, Ikenne) and water *ad libitum.* Mice were bred in plastic cages with wood chip beddings at the Animal House, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne. Beddings in the cages were ensured to be dust-free, non-toxic and free of pathogenic organisms. The beddings were changed every other day to keep the cages clean and hygienic. Mice were acclimatized for four weeks before the commencement of experimental protocols.

Experimental Design And Grouping

Forty male mice (40) were assigned into groups using body weight randomization techniques, animal were randomly divided into four (4) groups with ten (10) mice in each group. Labeled A, B, C and D

Group A: received 14.3ml/kg of distilled water.

Group B: received 14.3ml/kg of fresh palm wine everyday

Group C: received 14.3ml/kg of fresh palm wine at a day interval

Group D: received 14.3ml/kg of 50% diluted fresh palm wine at a day interval.

MEASUREMENT OF BODY WEIGHT

The animal's body weight were measured using a sensitive weighing balance during acclimatization period, once in a week throughout the period of experiment using sensitive weighing scale to assess the weight gain or loss in each group.

Rectal Temperature

The mice rectal temperature were examined thrice in a week throughout the period of experiment using digital thermometer to examine if there are any changes in the body temperature in each group.

Locomotor Activities

Animals were subjected to behavioural protocol associated with motor functions; behavioural studies were performed once in a week before and after administration of palm wine.

Neurobehavioural Studies

The neurobehavioural studies were carried out by using Open field test and it was performed once in a week.

OPEN FIELD TEST

This was used to assess the locomotory and exploratory activities in experimental mice. The behavioral parameter scores include:

a. Line Crossing: Frequency with which the mice crossed one of the grid lines with all four limbs measure locomotor activity.
b. Center Square Entries: Frequency with which the mice crossed one of the red lines with all four limbs into the central square measure exploratory and anxiety.
c. Rearing: Frequency with which the mice stood on their hind limbs in the maze measure locomotor activity.
d. Grooming: Number of times the animal spent licking or scratching itself while stationary.
e. Walling: Frequency with which the mice rear against the wall on their fore limbs.
f. Urination: Number of puddles or streaks of urine left behind and it measure anxiety.
g. Defecation: Number of fecal boli left behind and it measure anxiety.

Animal Sacrifice

Twenty four hour after the last administration, the animals were anaesthetized using chloroform. Tissues were excised by dissecting the head and the skulls of the mice were opened up and the cerebellum was carefully dissected out and weighed using sensitive weighing scale (Atomic scientific weighing scale). Tissue was quickly fixed in 10% formal saline (made from 8.5% of Nacl, 100ml of formaldehyde and 900ml of distilled water). This preserved the tissues and protected it against subsequent processing steps for routine histological study.

RELATIVE ORGAN WEIGHT

The relative organ weight of each animal was calculated as follows:

$$\text{Relative Organ Weight [ROW]} = \frac{\text{Organ weight (g)} \times 100}{\text{Body weight of mice on sacrifice day (g)}}$$

TISSUE PROCESSING

Following fixation, the tissues were processed for paraffin wax embedding. This is the most commonly used embedding medium in both normal and pathological histology. The recommended procedure was adopted (Bancroft, J., & Gamble, M. 2008).

The tissues were dehydrated through a series of graded ethanol (alcohol) solutions at an interval of one hour per change; most of the water in specimens must be removed before it can be infiltrated with wax at a later stage of processing.

50% ethanol----------1 hour
70% ethanol ----------1 hour
90% ethanol ----------1 hour
95% ethanol ----------1 hour
100% ethanol----------1 hour
100% ethanol----------1 hour

Dehydrated tissues were cleared in xylene as follows:

Xylene I----------1 hour
Xylene II----------1 hour

The tissues were infiltrated in two changes of molten wax at 60°C in the oven for one hour each and finally embedded in paraffin wax using plastic embedding moulds smeared with glycerin so the paraffin blocked tissues can be separated from the mould after embedding.

Paraffin blocked tissues were trimmed and mounted on wooden blocks for sectioning on a rotary microtome. Sections of 6µm thickness were obtained on a rotary microtome. The sections were spread in warm bath, and collected on clean glass slides smeared with egg albumen. The slides were then dried on a drying plate at a temperature of 40°C overnight to enhance adherence, and stored in slide racks until ready for staining.

Haematoxylin and Eosin method was used to demonstrate the general histoarchitecture of the cerebellum. Cresyl Fast Violet technique was used for demonstration of Nissl substance. Bielschowkey's staining method was used for demonstration of Axon and dendrites.

Photomicrography

These sections were examined under Accuscope LCD microscope interface with a digital camera was used for the light microscopic examination of all stained sections; Photomicrographs of stained sections were taken at various magnifications.

STATISTICAL ANALYSIS

All data were subjected to descriptive and inferential statistics. All values were expressed in M ± SEM using One-way analysis of variance (ANOVA) with P<0.05, considered statistically significant.

RESULT

Animal Body Weight (G)

Fig 1 shows that the mean body weight of animals at the commencement of the experiment was 22.24±3.83. Throughout the experiment, all the animals in the control group increased progressively in weight from 21.86 ±1.18 to 27.72±0.65(~10.74% increase) to the end of the experiment; a total of 5.86g was the body weight gain. However, it was not so for other groups. During the period of acclimatization, there was a progressive increased in body weight gain of treated groups but from the commencement of the administration to end of the experiment there was decrease in the body weight of the entire treated group. At end of the experiment the changes in body weight were compared to the control and it was observed to be statistically not significant (p>0.05).

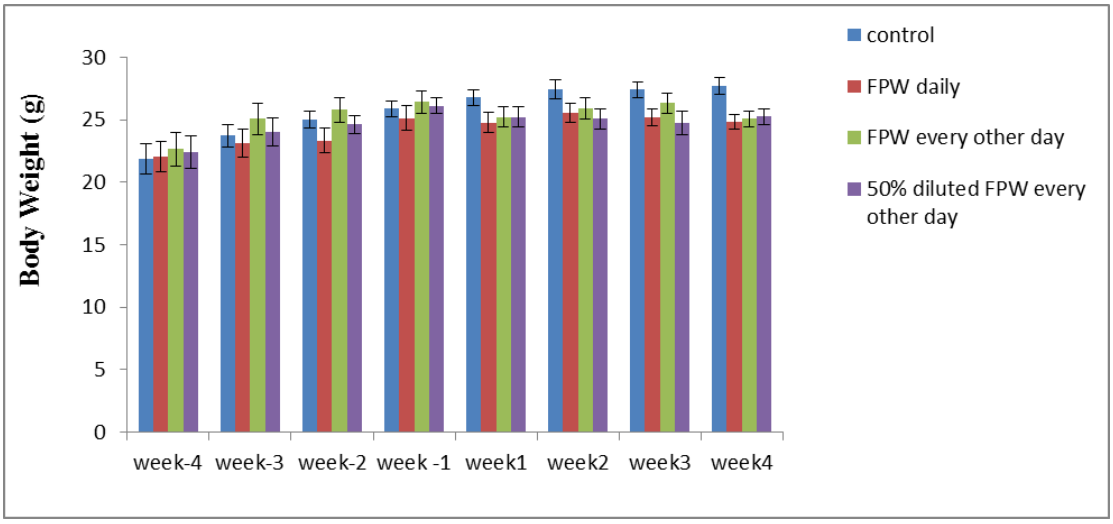

Figure 1: Bar chart of the effect of palm wine on the Body weight. Data are expressed as Mean± SEM. (n=10, p<0.05)

Rectal Temperature

Fig 2 shows the rectal temperature of mice before, during and after the administration of palm wine. During the period of acclimatization there was no tangible variation between the groups aside from the second week of acclimatization that there was increase in the rectal temperature of group C (50% diluted FPW every other day) which was due to the disarrangement of the cages. At the end of the four weeks of administration there were decrease in the rectal temperature of the entire treated group but that of group B (34.84±0.12) which received FPW daily were lower compared to group A (control group). At the end of experiment it was significant (p<0.05).

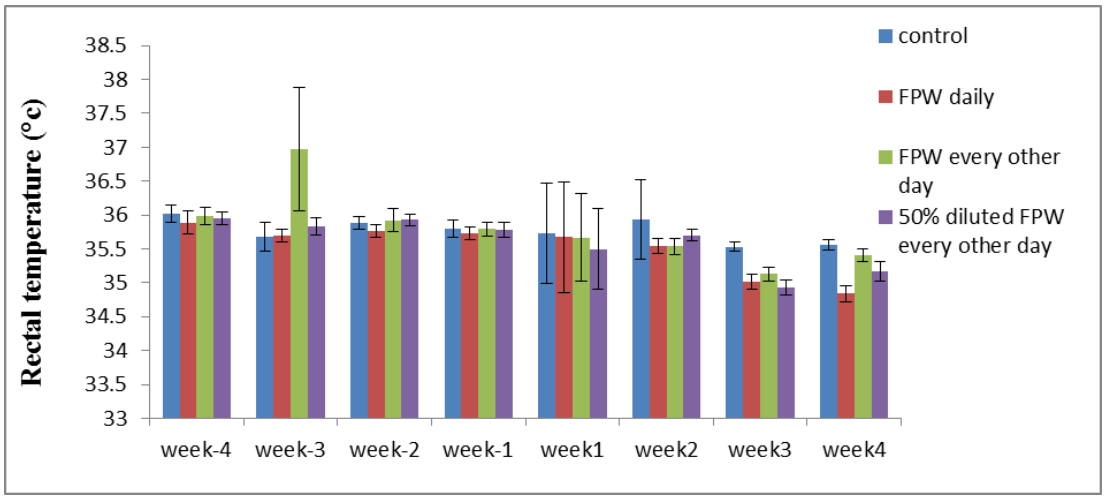

Figure 2: Bar chart of the effect of palm wine on the rectal temperature. Data are expressed as Mean± SEM. (n=10, p<0.05).

NEUROBEHAVIOURAL STUDIES

Linecrossing

Fig 3 shows that during the period of acclimatization there was no change in variation of line crossing in the entire groups. However, there were reduction in the frequency of line crossing of control group when compared with the treated groups in the first two weeks of administration but at the end of the study the frequency of line crosses was significantly lower ($p<0.05$) in the groups treated with FPW daily (36.50±7.48), FPW every other day (40.10±7.28) and 50% diluted FPW every other day (38.80±7.78) compared with the control (48.00±1.64).

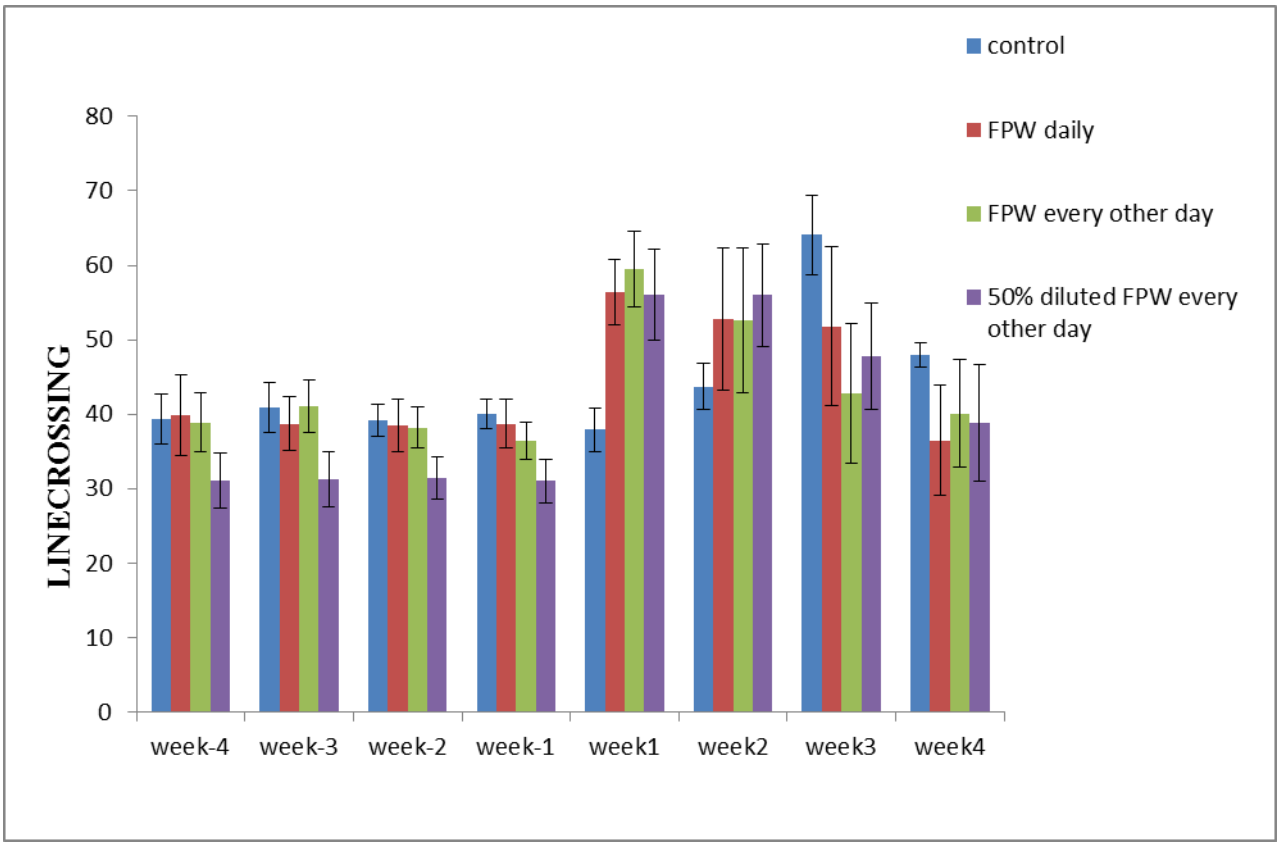

Figure 3: Bar chart of the effect of palm wine on line crossing. Data are expressed as Mean± SEM. (n=10),P<0.05.

REARING

Fig 4 shows that during the period of acclimatization the difference between the treated groups compared to the control is not significant. However, after the first week of administration there was increase in the frequency of rearing of the treated group compared to control but at the end of study there were reduction in the rearing frequency and it is significantly lower in the treated groups [FPW daily (5.80±1.18), FPW every other day (6.90±1.33) and 50% diluted FPW every other day (7.70 ± 1.76) compared to control group (12.20±1.31)

.

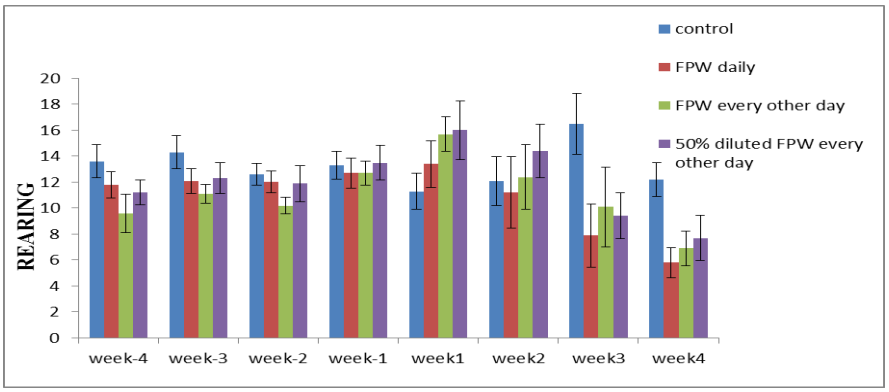

Figure 4: Bar chart of the effect of palm wine on rearing. Data are expressed as Mean± SEM. (n=10) (P<0.05).

WALLING

Fig 5 shows the walling frequency in the open field test. There was no significant difference during the period of acclimatization when the treated groups were compared with the control. The frequency of walling is significantly lower ($p<0.05$) in the groups treated with FPW daily (7.90±1.60), FPW every other day (8.80±1.58) and 50% diluted FPW every other day (9.90±2.19) compared to control group (16.70±1.48).

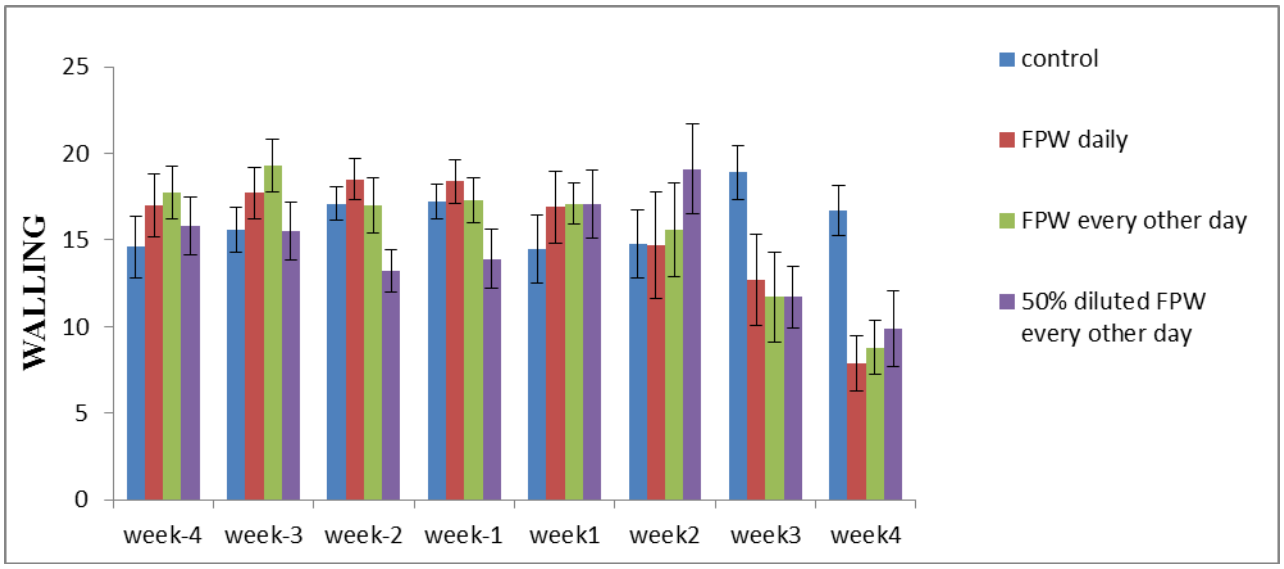

Figure 5: Bar chart of the effect of palm wine on walling. Data are expressed as Mean± SEM. (n=10) ($P<0.05$).

GROOMING

Fig 6 shows the grooming frequency in the open field test. There was no significant difference between the three treated groups and control during the period of acclimatization. However, after the third week of administration there was increase in the grooming frequency of FPW every other day and 50% diluted FPW every other day compared to FPW daily and control group. At the end of study there was no significant difference between the treated groups[FPW daily (7.10±1.57), FPW every other day (5.60±1.10) and 50% diluted FPW every other day (6.20±1.35)] and the control (9.80±1.70).

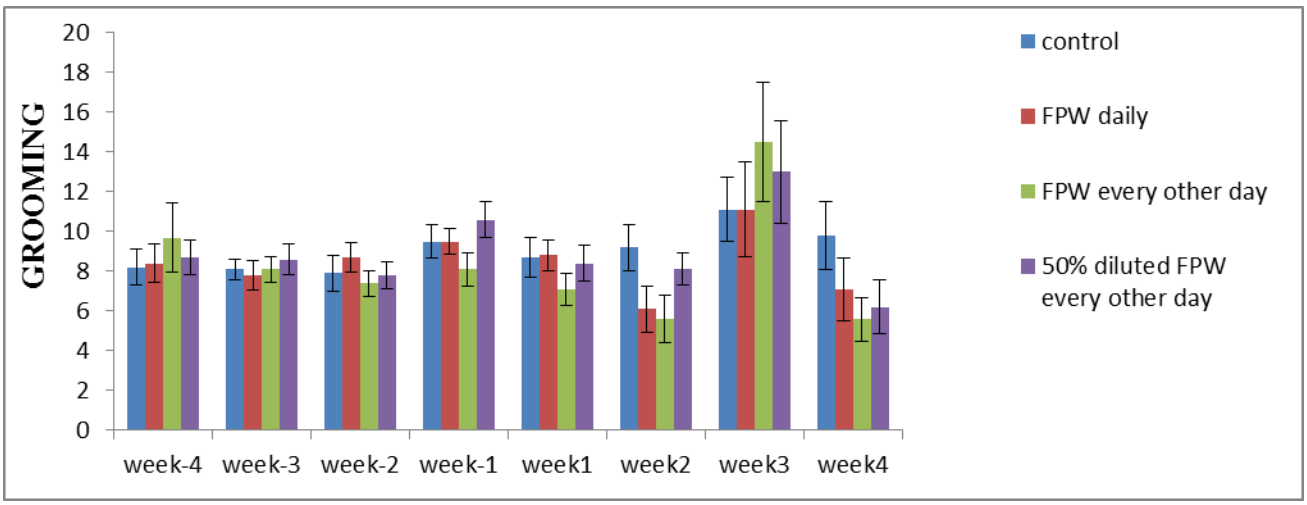

Figure 6: Bar chart of the effect of palm wine on grooming. Data are expressed as Mean± SEM. (n=10) ($P<0.05$).

CENTRE SQUARE

Fig 7 shows the centre square frequency in the open field tests. There is no significant difference in centre square frequency in treated groups compared to the control during the period of acclimatization. However, centre square frequency at the end of the study was significantly lower in all the treated groups (FPW daily (0.30±0.21), FPW every other day (0.70±0.30) and 50% diluted FPW every other day (0.80±0.17) compared to control group (1.60±0.45).

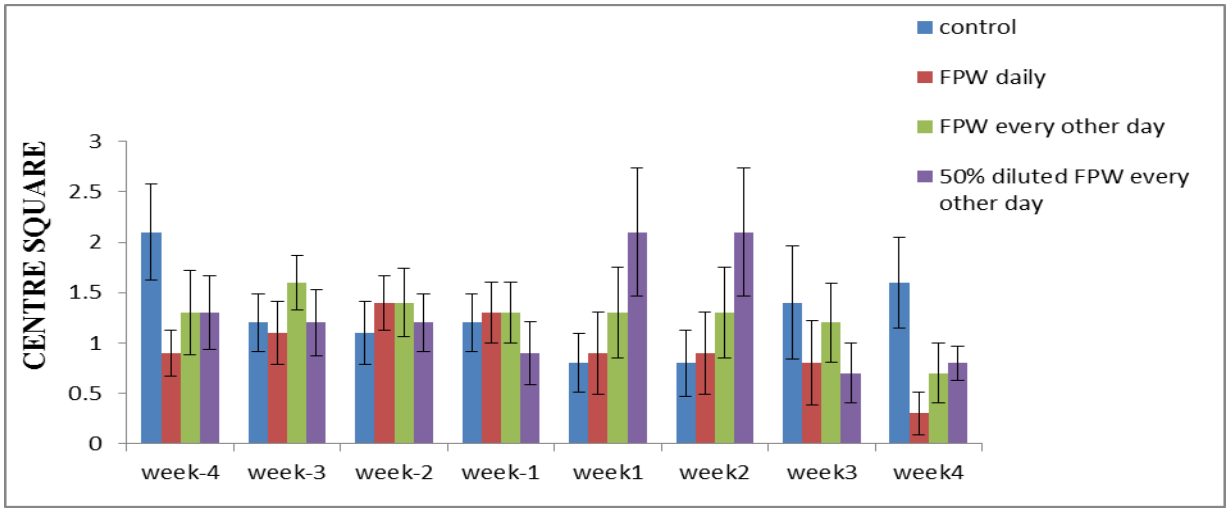

Figure 7: Bar chart of the effect of palm wine on grooming. Data are expressed as Mean± SEM. (n=10) (P<0.05).

DEFECATION

Fig 8 shows the defecation frequency in the open field test. There were no significant differences in the treated group compared to control group during the period of acclimatization and administration.

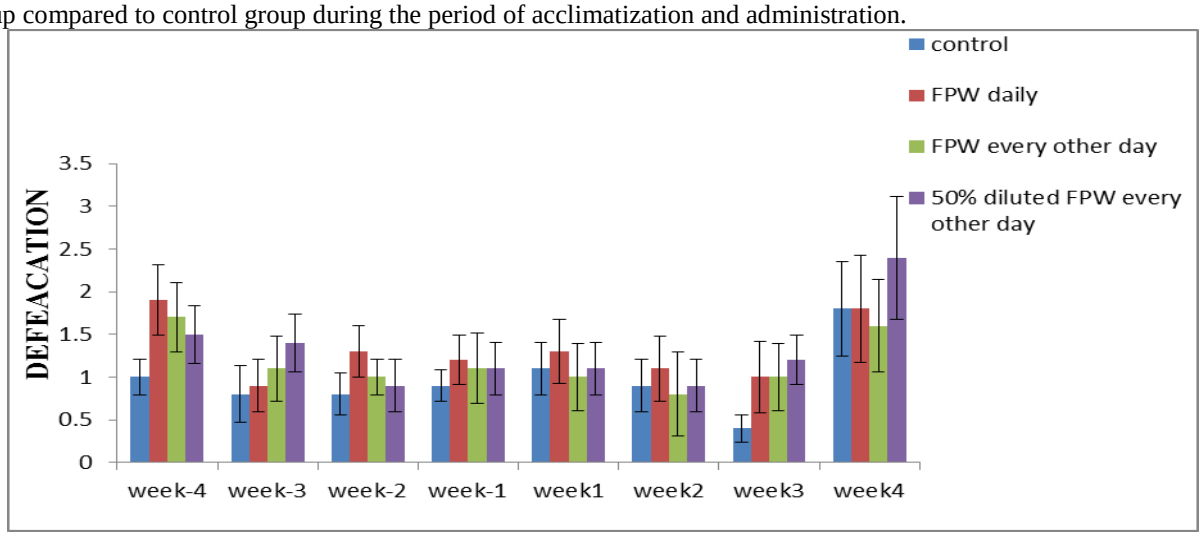

Figure 8: Bar chart of the effect of palm wine on defecation. Data are expressed as Mean± SEM. (n=10) (P<0.05).

URINATION

Fig 9 shows the urination frequency in the open field test. There were no significant differences in the treated group compared to control group during the period of acclimatization and administration.

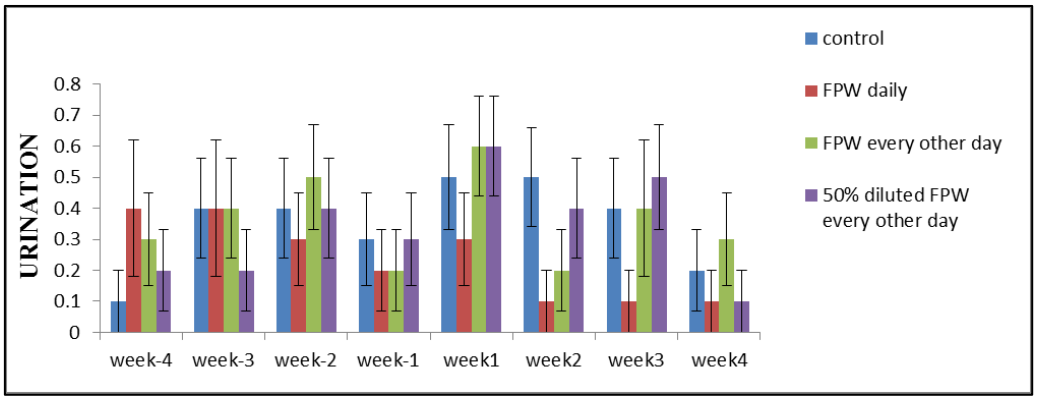

Figure 9: Bar chart of the effect of palm wine on urination. Data are expressed as Mean± SEM. (n=10) (P<0.05).

RELATIVE ORGAN WEIGHT

Fig 10 shows the cerebella weight in all experimental groups and there was increase in their relative organ weight compared to the control and the increase was statistically significant. Group B that take palm wine everyday was the highest (0.1690 ± 0.0088) when compared with the control (Group A) (0.1280 ± 0.0042) and other two experimental groups (group C: 0.1560 ± 0.0127, group D: 0.1630 ± 0.0149)

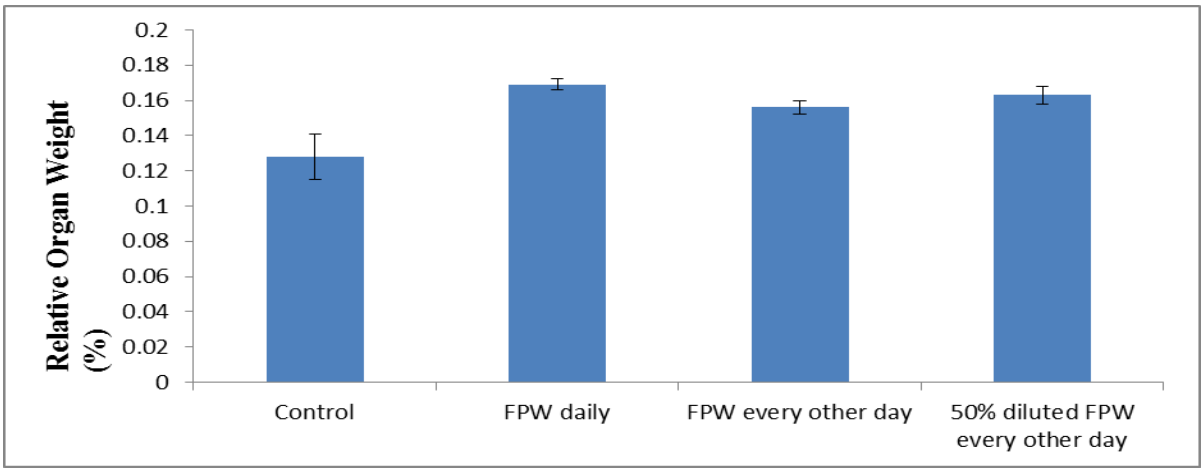

Figure 10: Bar chart of the effect of palm wine on relative organ weight. Data are expressed as Mean± SEM. (n=10) (P<0.05).

Histopathological Findings

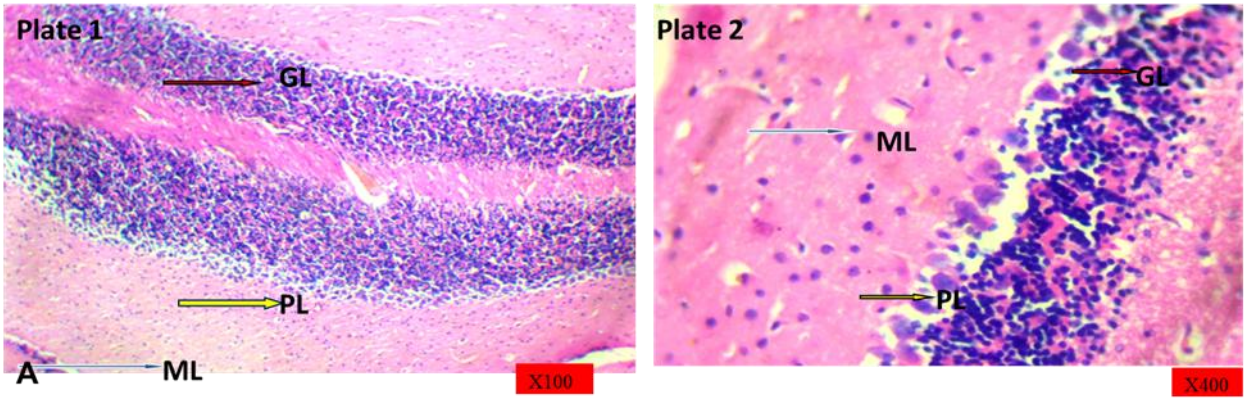

Figure 11: Photomicrograph of group (A) (Control group) x 100 and x 400

Plate 1: Photomicrograph of cerebella of cortex Control (Group A) (H&E x100). It shows a normal cerebella cortex with the external molecular (blue arrow), middle Purkinje (yellow arrows) and inner granular cell (red arrow) were well defined. Plate 2: Photomicrograph of cerebella cortex of Control (Group A) (H&E x400). Photomicrograph at higher magnification, the three layers of the cerebella cortex was clearly observed as well as its constituent cells. Molecular layer showing stellate and basket cells (blue arrow); Purkinje layer showing Purkinje cell bodies (yellow arrows); Granular layer showing granule cell (red arrow)

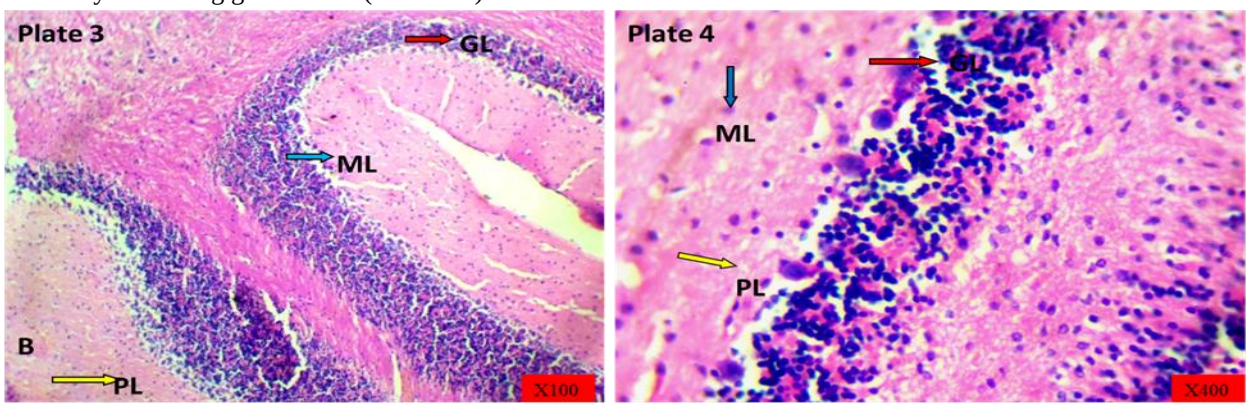

Figure 12: Photomicrograph of group (B) (Fresh palm wine everyday) x 100 and x 400

Plate 3: Photomicrograph of cerebella cortex of group B (Fresh palm wine everyday) (H&E X100).It presented a cerebella cortex with all layers intact and cell well demonstrated. Plate 4: Photomicrograph of cerebella cortex of group B (Fresh palm wine everyday) (H&E X400). The cells are well defined, the cell of granular layer at the higher magnification appeared more compact and it may enhance cell to cell communication. It shows layout of the cerebella cortical layers: molecular (blue arrow), Purkinje (yellow arrow) and granular (red arrow).

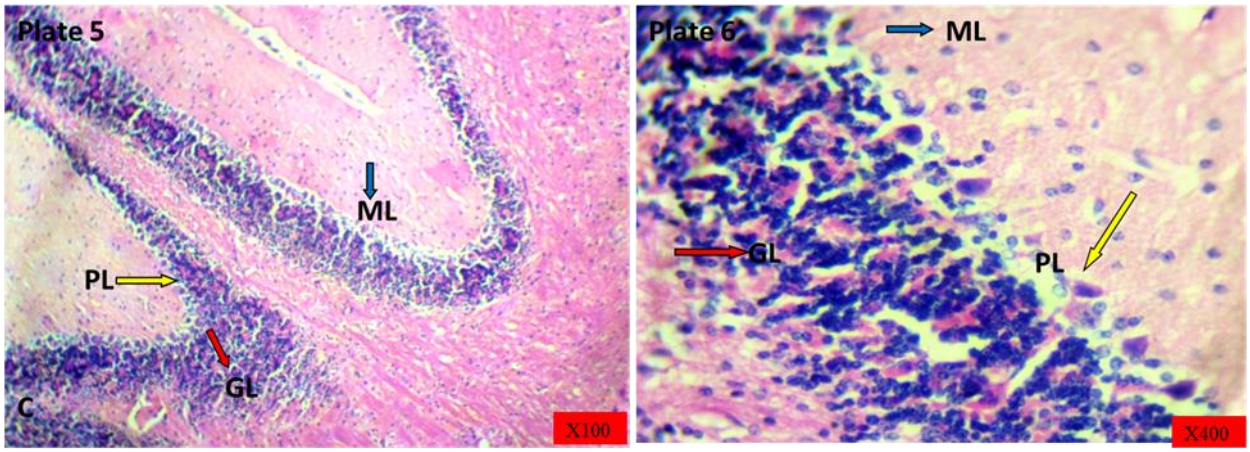

Figure 13: Photomicrograph of group (C) (Fresh palm wine every other day) x 100 and x 400

Plate 5: Photomicrograph of cerebella cortex of group C (Fresh palm wine every other day) (H&E X100). The layers of the cerebella cortex are clearly seen and their cells were well defined. Plate 6: Photomicrograph of cerebella cortex of group C (Fresh palm wine every other day) (H&E X400). The cerebella cortex of the group C (FPW every other day) animals showed the general histoarchitecture and its cellular constituent. Molecular (blue arrow), Purkinje (yellow arrow) and granular (red arrow).

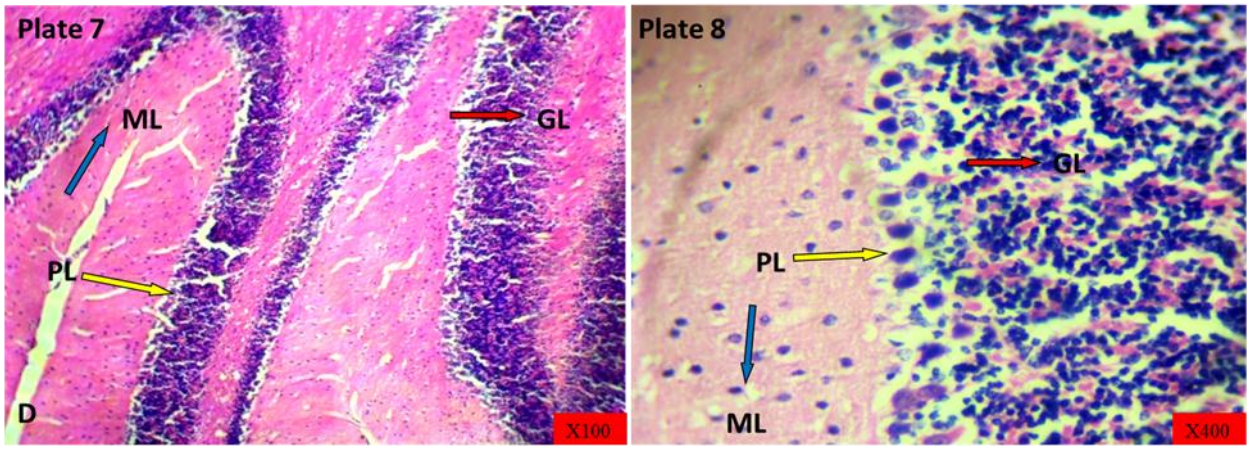

Figure 14: Photomicrograph of group (D) (50% diluted palm wine every other day) x 100 and x 400

Plate 7: Photomicrograph of cerebella cortex of group D (50% diluted fresh palm wine every other day) (H&E X100). Animals in group D have a preserved cerebellar cortex that is largely similar to the control. Plate 8: Photomicrograph of cerebella cortex of group D (50% diluted fresh palm wine every other day) (H&E X400). At higher magnification, there is relatively abundance of Purkinje cells and this could be a pointer to enhance function of this cells. It shows cerebellar cortical layers: molecular (blue), Purkinje (yellow) and granular (red).

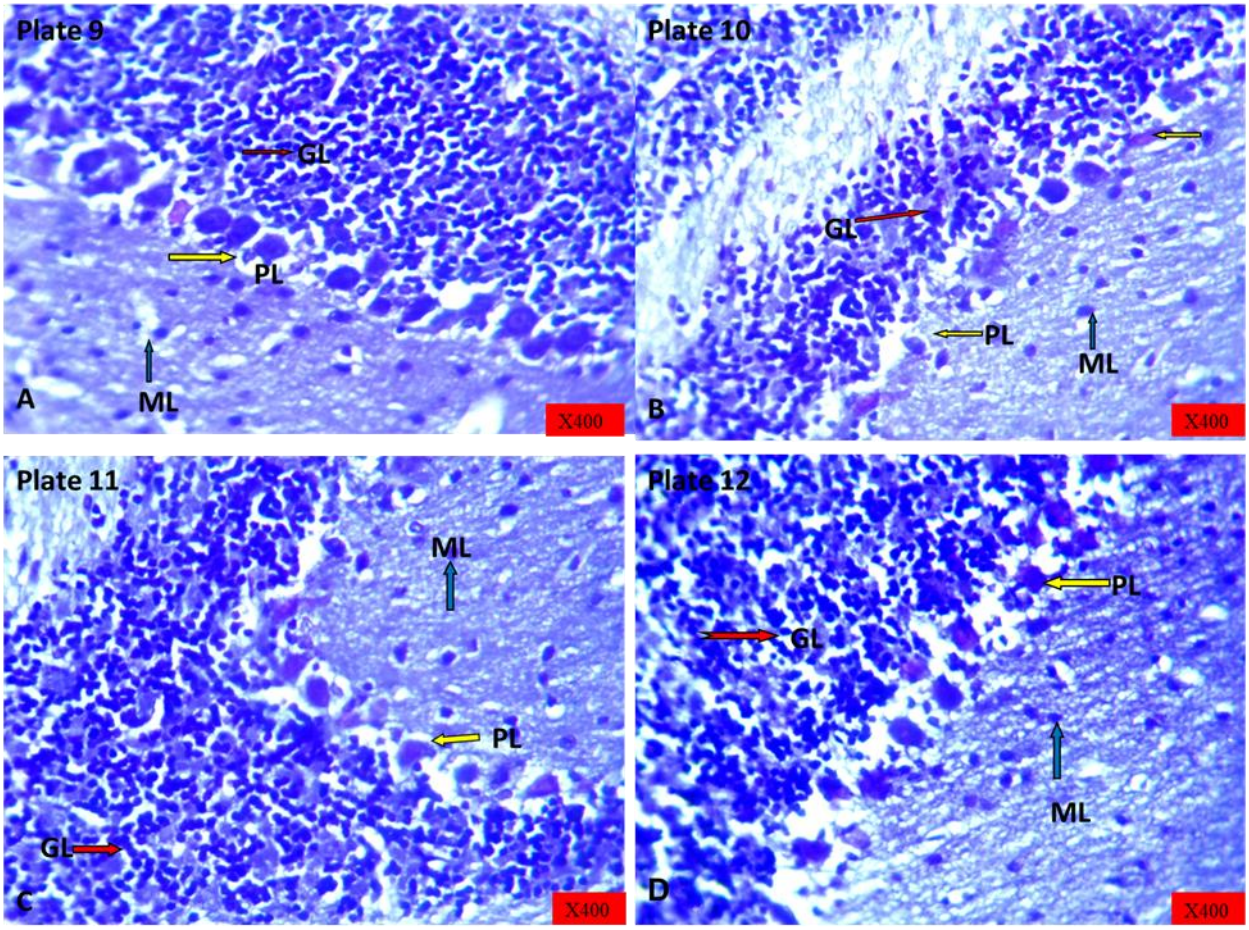

Figure 15: Photomicrograph of group (A) (Control group) X400, group (B) (Fresh palm wine everyday) X400, group (C) (Fresh palm wine every other day) X400 and group (D) (50% diluted palm wine every other day) X400

Plate 9: Photomicrograph of cerebella of cortex Control (Group A) (NISSL x100). It stained adequately for Nissl material with the cell adequately demonstrated in the three layers. Molecular layer (blue arrow) showing stellate and basket cells; Purkinje layer (yellow) showing Purkinje cell bodies (green arrows); Granular layer (red) showing granule cell Plate 10: Photomicrograph of cerebella cortex of group B (Fresh palm wine everyday) (NISSL X100). The cell also stain adequately for Nissl material across various layers and Molecular layer (blue arrow) showing stellate and basket cells; Purkinje layer (yellow) showing swelling/ slight degeneration of Purkinje neuron cell bodies (green arrows); Granular layer (red) showing granule cell. Plate 11: Photomicrograph of cerebella cortex of group C (Fresh palm wine every other day) (NISSL X400). Molecular layer (blue arrow); Purkinje layer (yellow) showing vacuolations and slight degeneration, Purkinje neuron cell bodies (green arrows) and Granular layer (red) showing granule cell Plate 12: Photomicrograph of cerebella cortex of group D (50% diluted fresh palm wine every other day) (NISSL X400). These group animal stains relatively intense for Nissl material and the Purkinje cells in particular are prominently demonstrated.

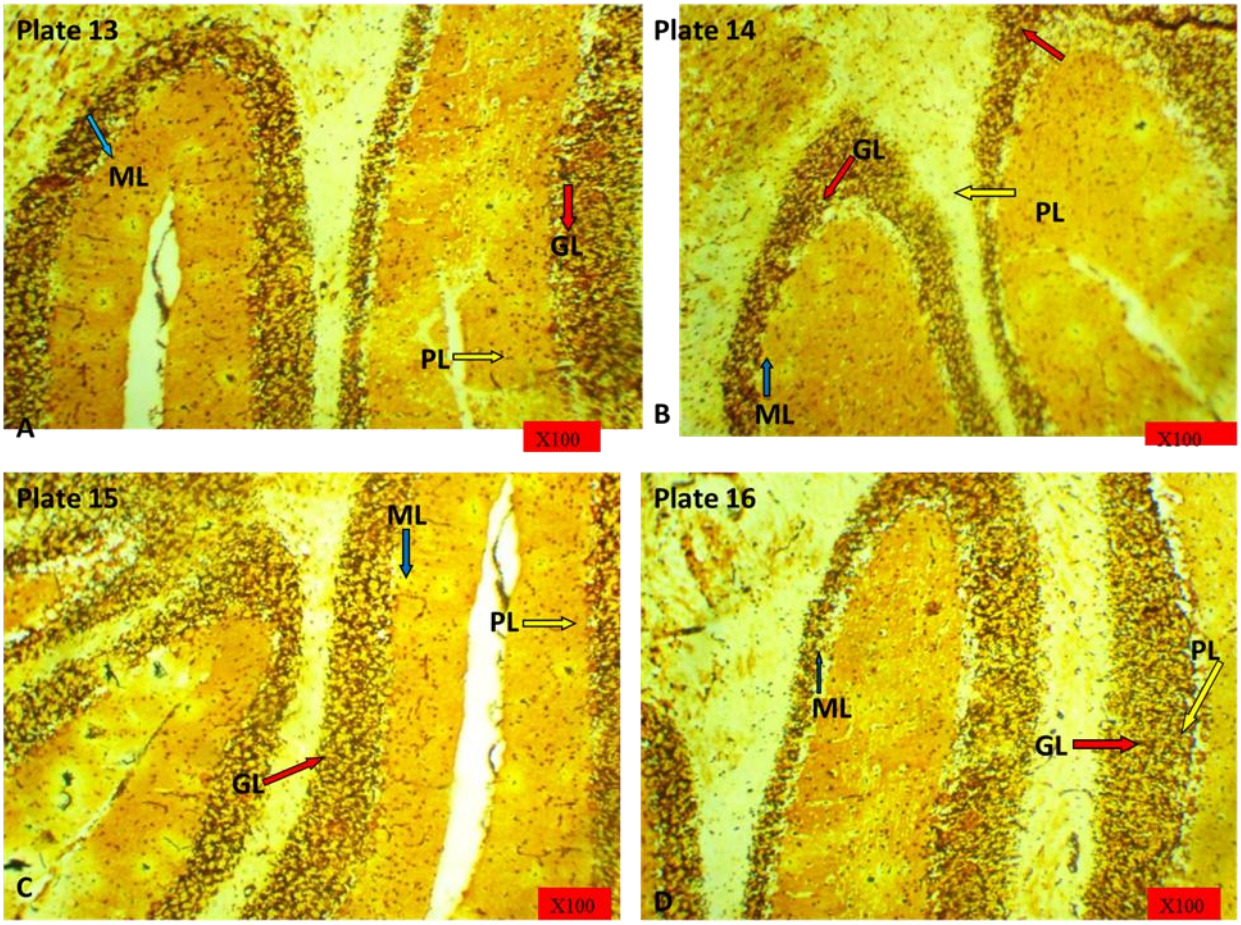

Figure 16: Photomicrograph of group (A) (Control group) X100, group (B) (Fresh palm wine everyday) X100, group (C) (Fresh palm wine every other day) X100 and group (D) (50% diluted palm wine every other day) X100

Plate 13: Photomicrograph of cerebella cortex of Control (Group A) (BIELSCHOWKY'S X100) that received distilled water only showed normal histological features of the molecular (blue arrow), Purkinje (yellow arrow) and granular (red arrow) layers.

Plate 14: Photomicrograph of cerebella cortex of group B (Fresh palm wine everyday) (BIELSCHOWKY'S X100). Molecular layer (blue arrow), Granular layer showing neuronal degeneration (red arrow) and Purkinje layer (yellow arrow) showing vacuolation.

Plate 15: Photomicrograph of cerebella cortex of group C (Fresh palm wine every other day) (BIELSCHOWKY'S X100). Molecular layer (blue), Purkinje layer (yellow arrow) showing vacuolations, Granular layer (red arrows) showing granule cells. Plate 16: Photomicrograph of cerebella cortex of group D. (50% diluted fresh palm wine every other day) (BIELSCHOWKY'S X100). Molecular layer (blue), Purkinje layer (yellow arrow), and Granular layer (red arrows) showing granule cells.

DISCUSSION

In this study, 40 mice were employed to investigate the histomorphological studies of the cerebella cortex and the behaviour of mice following palm wine administration.

The animals were acclimatized for four weeks and their body weights were taken periodically. At the end of the fourth week, there was uniform increase in the weekly mean body weights throughout the period of acclimatization for all groups. There was a decrease in the mean bodyweight of all experimental animals (FPW daily, FPW every other day and 50% DFPW every other day) at the end of the fourth week of administration of palm wine when compared with the fourth week mean bodyweight of acclimatization but there were no statistical variations when compared with the control. The decrease in the body weight could be as a result of palm wine causing reduction in food intake of the animals, since palm wine is basically made up of glucose and lactose, it could be the reason why lover of palm wine experience reduction of food intake since palm wine supplies energy to their system (Obire, O. 2005; & Chandrasekhar, K. *et al.*, 2012). No significant weight reduction was observed in all the experimental

animals groups when compared to the control group, the result obtained was consistent with similar observation reported by previous researchers that palm wine caused non-significant changes in body weight of rats after treatment for 30 days (Oyedeji, K. O. *et al.*, 2013).

Rectal temperature is use in the assessment of animal health status and obtaining animal body temperatures (Chung, T. H. *et al.*, 2010). Motor activity was a positive predictor of body temperature, which is consistent with many previous studies (Gordon, C.J. 1993; Gordon, C.J., & Yang, Y. 1997; Kent, S. *et al.*, 1991; & Refinetti, R. 1994). There is significant need for body temperature measurement in animals used for research because it is an overall marker of animal health and a prognostic indicator (Molins, C. R. *et al.*, 2012). The core body temperature is the measurement of deep body sites (Gordon, C.J., & Yang, Y. 1997). The rectal temperature of the entire animals was examined throughout the study. During the four weeks of administration of palm wine the treated groups were compared with control group. Result obtained from the first week of administration of palm wine showed reduction in the treated groups (FPW daily, FPW every other day and 50% diluted FPW every other day) when compared with control group. However, it was not significant compared to control. The rectal temperature from the 2nd to 4th (end of study) week of administration shows significant reduction in the treated groups when compared with control group.

The present study also evaluated the effect of palm wine on the behavior in mice using the open field test. The result obtained from the open field test revealed a significant ($p<0.05$) decrease in frequencies of rearing, line crosses, walling and centre square in the test groups compared to control. A decrease in these parameters indicates low locomotor exploration and conversely increased anxiety in the test animals compared to control. Thus, this present study revealed that administration of palm wine impaired the mice motor output and exploratory drive. Observation of the frequency of line crossing of the entire experimental animals in 1st and 2nd week of administration showed reduction in the control group when compared to treatment groups. The 3rd and 4th weeks of administration of palm wine showed a significant reduction in the frequency of line crossing in all treated groups when compared to control group. Observation after the first week of administration revealed an increase in rearing frequency of treated groups compared to control. However, the increase was statistically insignificant ($p>0.05$). The difference that occurred in rearing between the control and test animals were only significant ($p<0.05$) at (week 4) towards the end of the study. Similar result was reported by Nku *et al.*, 2015 in rats treated with Cannabis, Cataflam and Aspirin.

There was increase in the frequency of walling of the treated group during the 1st and 2nd weeks of administration. However, at the end of the study (4th week) there was reduction in frequency of walling and it was significantly lower in the treated groups compared to control. This result is consistent with the centre square frequency where there was a significant reduction in the frequency of centre square of treated group when compared to control.

Other neurobehavioural activities carried out were on grooming, defecation and urination. At the end of the study there was an increase in the grooming frequency of the control compared to treated groups, though not statistically significant ($p<0.05$).Defecation and urination frequency, however, tells a different story, there was fluctuation in defecation and urination frequency from the first week of administration to the end of study (4th week).

The analyses of the photomicrograph of animal group A to D were done following the principle of Garman 2012. Histological and morphological attributes including General Histomorphological integrity, Cytoarchitectural integrity and Cell fibre integrity were considered. Furthermore, these parameters enable the observation of cell morphology, cell spatial distribution, Nissl substance demonstration and possible presence of associated anomalies to be observed. Suitable magnification where selected; presenting the general histoarchitectural overview (At the lower magnification X100) and detailed histological element (At the higher magnification X400).

Histomorphological integrity of the cerebella cortex in the Photomicrograph of the group A (control group) animals (Fig 11) at the lower magnification present a normal cerebella cortex with the external molecular layer, middle Purkinje layer and inner layer well defined, cell population and spatial distribution also indicate that the cortex is normal. At the high magnification the three layers are clearly observable as well as the constituent cells.

The photomicrograph of cerebella cortex of group B (FPW daily) (Fig 12) presented cerebella cortex with all layers intact and cell well demonstrated. Molecular cells are demonstrated in the outer layer; so also the Purkinje cell between molecular and layer of granular cell. It is however interesting to know that the cell of granular layer at the higher magnification appeared more compact relative to spatial distribution while this may not necessary suggest a structural improvement over the group A (control group) cerebella cortex but there was mild observable destruction in the cell. The cerebella cortex of the group C (FPW every other day) animals (Fig 13) showed the general histoarchitecture and its cellular constituent. Notwithstanding the tissue did not also have any sign of structural enhancement to improvement relative to the

control group. The group D (50% diluted FPW every other day) animals (Fig 14) also have preserved cerebella cortex that is largely similar to the control. However there is relatively prominent or abundance of the Purkinje cells. This observation suggest that this cells have relatively more elaborate morphology and relative abundance, this could be a pointer to enhance function of this cells, since the Purkinje cells are primary output cells the elaborate morphology of this could imply reinforce or enhance cerebella output which could possibly influence motor activity (Purves, D. *et al.*, 2001) also since these cell have regulator function this observation might also suggest normal cerebella function.

Cytoarchitectural Nissl integrity was observed using cresyl fast violet staining (Fig 15). This stain gives a better understanding of cytological conditions. The demonstration of Nissl substance neuron helps to appreciate the functional integrity of the cells; this is because the demonstrated Nissl material is primary active rough endoplasmic reticulum in conjunction with the associate ribosome. Thus, demonstration of the Nissl substance as well as it intensity gives useful information about the tissue structural and functional integrity. Cerebella cortex of control group stained adequately for Nissl material with the cell adequately demonstrated in the three layers. The cerebella cortex of the group B animal also present a demonstration, the cell also stain adequate for Nissl material across the various layer, there were mild structural aberration in this group showing swelling/ slight degeneration of Purkinje neuron cell bodies, this implies that the administer palm wine alter the structural and functional integrity of cerebella cortex in this group. The cerebella cortex of group C animals also showed vacuolation and slight degeneration of Purkinje neuron cell bodies. Relative to the other groups the cortical element of the group D animal's stain relatively intense for Nissl material and the Purkinje cells in particular are prominently demonstrated. Their prominence and relative distribution also suggest relative abundant , in even the neutrophil in the molecular layer stained relative intense, bearing in mind the function and important of the Nissl material this observation suggest a mild functional enhancement of the cortical element in this group relative to other group, this suggest that the administer palm wine might not be deleterious in group D, it appears to have a mild positive effect on cortical, structural and functional integrity, thus the dosage and frequency of administration appear to be important factors in determining the nature of the effect of palm wine as use in the current investigation.

Cell and cortical fibre integrity was observed using the Bielschowsky (silver) stain techniques demonstrate cell morphology and cortical fibrils in addition (Fig 16). The photomicrograph of group B revealed swelling and degeneration of Purkinje neuron cell bodies and group C showed slight degeneration of Purkinje neuron cell bodies. This method shows that cell morphology was altered. This observation indicated that administered palm wine altered the cortical histoarchitecture.

Hence, there were observable effects which were vacuolation and slight degeneration of Purkinje neuron cell bodies in all treated group except group D that took 50% diluted FPW every other day with a preserved layers(granular, Purkinje and molecular layer).

Organ weight changes have long been accepted as a sensitive indicator of chemically induced changes to organ. Comparison of organ weights between treated and untreated groups of animals have conventionally been used to evaluate the toxic effect of the test article (Peters, J.M., & Boyd, E.M. 1966; & Pfeiffer C.J 1968). The relative organ weight (%) of the mice after oral administration of palm wine is presented in figure 16. The relative weights of cerebellum was significantly higher ($p<0.05$) in the groups that received palm wine compared to control. Result obtained showed that cerebellum of treated groups increased in weight. The result from this study was in agreement with Nayanatara *et al.,* 2009, It is important to state that the significant ($p<0.05$) differences in the organ weight of male mice could be attributed to the presence of high carbohydrate (glucose and fructose) intake in the treated mice groups (Shamala, T.R., & Srikantiah, K.R. 1988).

This present study was conducted and financed by the authors without any external sponsors from any firm or organization.

REFERENCES

1. Agbeniyi, S.O., & Ayodele, M.S. (2010), *Palastan journal of Nutrition*, 9, 512-515.
2. Agu, R. C., OKENCHP, M., Ude, C. M., ONY, A., ONWMELU, A., & AJIW, V. (1999). Fermentation Kinetic Studies. *J. Food Sci. Technol*, *36*(3), 205-209.
3. Bancroft, J., & Gamble, M. (2008). Theory and practice of histological techniques. 6th Edition published by Church livingston Elesevier
4. Bell C.C, Han, V., & Sawtell, N.B. (2008), *Annual Reviewed Neuroscience*.,31, 1–24
5. Chandrasekhar, K., Sreevani, S., Seshapani, P., & Pramodhakumari, J. (2012). A review on palm wine. *International Journal of Research in Biological Sciences*, *2*(1), 33-38.
6. Chung, T. H., Jung, W. S., Nam, E. H., Kim, J. H., Park, S. H., & Hwang, C. Y. (2010). Comparison of rectal and infrared thermometry for obtaining body temperature of gnotobiotic piglets in conventional portable germ free facility. *Asian-Australasian Journal of Animal Sciences*, *23*(10), 1364-1368.
7. Faparusi, S. I. (1981). Sugars identified in Raphia palm-wine. *Food chemistry*. *7*(21), 81-3

8. Garman H.R (2012). Histology of the central nervous system. *Toxicologic pathology* 39
9. Gordon, C.J. (1993). Temperature regulation in laboratory rodents. *Cambridge University Press, New York.*
10. Gordon, C.J., & Yang, Y. (1997). Contribution of spontaneous motor activity to the 24 hour control of body temperature in male and female rats. *Journal of Thermal Biology* 22, 59-68.
11. Hamre, K., & Goldowitz, D. (1998). The cells and molecules that make a cerebellum, TINS 21 375–382.
12. Kent, S., Hurd, M., & Satinoff, E. (1991). Interactions between body temperature and wheel running over the estrous cycle in rats. *Physiology & Behavior* 49, 1079-1084.
13. Martinez, M. C., Depinto, G. L., & Rivas, C. (1992). Composition of Acacia MacracanthaGum Exudates. *Phytochemistry*, *31*(2), pp. 535-536.
14. Molins, C. R., Delorey, M. J., Young, J. W., Yockey, B. M., Belisle, J. T., Schriefer, M. E., & Petersen, J. M. (2012). Use of temperature for standardizing the progression of Francisella tularensis in mice. *PLoS One*, *7*(9), e45310.
15. Nayanatara, A. K., Nagaraja, H. S., Ramaswamy, C., Bhagyalakshmi, K., Bhat, M. R., & Harini, N. (2009). Estimation of tissue lipid peroxidation level and organ weight in litters of wistar rats exposed to prenatal alcohol ingestion. *J Physiol Biomed Sci*, *22*, 44-47.
16. Ndon, B.A. (2003). The Raffia palm. 1st Ed. p.16. Concept Publications Ltd, Lagos, Nigeria
17. Nku, C.O., Ogbalie, G.O., & Ajiwhen, I.O. (2015). Locomotor behaviour and anxiety in the open field and light/ dark box in CD1 mice treated with aspirin, cataflam and ethanolic extract of cannabis sativa. *British journal of medicine and medical research 6*,232-564.
18. Noll, R.G. (2008). The wines of West Africa: History, technology and tasting notes. *Journal of Wine Economics*; 3:85-94.
19. Obire, O. (2005). Activity of Zymomonasspecies in palm sap obtained from three areas in Edo state, *Nigeria Journal of Applied Science Environental Management* ;9: 25-30.
20. Okafor, N. (1978). Microbiology and Biochemistry of oil palm wine. *Advanced Applied Microbiology:* 24*:*237 –254.
21. Okpatu. (2013). African Palm Wine - a Different Type of Wine
22. Otedoh, M. O. (1990). Sweet Raffia palm Wine. *The Nigerian Field*. 55, pp. 59 – 64.
23. Oyedeji, K. O., Bolarinwa, A. F., & Fashina, A. M. (2013). Effect of Alcohol consumption on haematological and reproductive parameters in female albino rats, *Journal of Dental and Medical sciences 3*(5), 76-79
24. Peters, J.M., & Boyd, E.M. (1966). Organ weight and water levels of the rat following reduced food intake. *Journal of nutrition 90*(4),354-360.
25. Pfeiffer C.J (1968) A mathematical evaluation of the thymic weight parameter. *Toxicological and applied pharmacology* 13(2):220-227
26. Purves, D., Augustine, G.J., Fitzpatrick, D., Lawrence, C., James, O.M., & Mark, S.W. (2001). Neuroscience, 2nd edition Sundertland MA: Sinauer Associates Chapter 28
27. Refinetti, R. (1994). Contribution of locomotor activity to the generation of the daily rhythm of body temperature in golden hamsters. *Physiology & Behavior* 56, 829-831
28. Saab, C. Y., & Willis, W. D. (2003). The cerebellum: organization, functions and its role in nociception. *Brain research reviews*, *42*(1), 85-95.
29. Shamala, T.R., & Srikantiah, K.R. (1988).“Microbial and biochemical studies on traditional Indian palm wine fermentation”. *Food Microbiology*; *5*, 157-162.
30. Uraih, N., & Iwuagbe, Y. (1990). Public Health, Food and Industrial Microbiology. Uniben Press Nigeria. Pp. 149 – 164.