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EFFECTS OF SUB-ACUTE ADMINISTRATION OF FERMENTED *ELAEIS GUINEENSIS* SAP (PALM WINE) ON THE PRE-FRONTAL CORTEX OF ADULT WISTAR RATS

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Abstract: **Background: Palm wine (sap from *Elaeis guineensis*) is known to have alcoholic contents which increases upon fermentation, yet it is being consumed in large and chronic dimensions without strict government regulations. Aim: This study aimed at investigating the effects of sub-acute administration of fermented *Elaeis guineensis* sap (palm wine) on the pre-frontal cortex of the brain using adult wistar rats. Methodology: Unadulterated palm wine (*Elaeis guineensis* Sap) was diluted with 50% clean tap water, and allowed to ferment at room temperature for 24 hours before being administered to the rats. Twenty five (25)** healthy male wistar rats **with average weight of 200g were divided into five groups (n=5). Group A served as the normal controls and received only distilled water daily. Group B, C, D, and E were received increasing volumes of fermented palm wine via oral routes in increasing progressions (1ml, 2ml, 4ml, and 8ml respectively). The experimental procedure lasted for 14 days. 24 hours after their last administration, the rats were sacrificed under ketamine (100mg/ml) as anesthesia. Their brain mass were carefully harvested, the prefrontal cortices were isolated for histological analyses and stained with Cresyl Fast Violet and also Hematoxylin and Eosin. Results: Treated groups showed progressive dose-related injuries evidenced by vacuolation of neurons, dilation of perivascular spaces, fatty changes, neuronal atrophy and loss of nissl bodies. Severity of these effects increased as dosage increased. Conclusion: Fermented *Elaeis guineensis* Sap demonstrated a clear dose-dependent deleterious effects on the histomorphorlogy of the prefrontal cortex.**

***Keywords: Elaeis guineensis*, Alcoholic beverage, Pre-frontal cortex, Neurotoxicity.**

1. INTRODUCTION

Palm wine is a trending traditional alcoholic beverage commonly consumed by the multitude for either cultural, personal or ceremonial reasons among Nigerians. It is popularly consumed among the Igbos of the South -Eastern region of Nigeria and is the sap from oil palm (*Elaeis guineensis)* (Eluwa *et al.*, 2010).

It is milky in appearance and is a rich nutrient medium containing sugar, protein, amino acid, alcohol, vitamins and minerals (Ezeagu and Fatunso, 2003). It also contains a dense population of yeasts (Bassir and Maduagwu, 1978). These contents including its low alcoholic content, lactic acid, and acetic acid is known to confer probiotic qualities to the drink and is responsible for the health benefits attributed to the drink (Rakowska *et al.*, 2017; Nwaiwu and Chikezie, 2020).

Beverages are the most active functional food category but there are specific concerns over their safety (Tamang et al., 2016). Oil palm (*Elaeis guineensis)* sap is known to contain natural phenols (Nwaiwu *et al.*, 2016) and in vitro tests have shown antioxidant activity (Oboh and Okhai, 2012). However, the distinctiveness of specific phenols from palm wine are yet unknown (Nwaiwu and Chikezie, 2020). Ogunro and Ologunagba, (2011) recorded that palm wine depletes the body's antioxidants, which may allow free radicals to attack and subject the body to a state of oxidative stress. It is known to have alcoholic contents of which its Ethanol content (5%) varies as it increases during fermentation (Bassir, 1962; Osim *et al*., 1991; Nwaiwu and Chikezie, 2020).

It has been known that fermented palm wine has teratogenic effects on the histology of fetal cerebral cortex of wistar rats (Eluwa *et al*., 2010). The developing adolescent brain is particularly vulnerable to the toxic effects of alcohol (Guerri *et al*., 2010). In a study by Chignon *et al*. (1998), alcohol misuse is associated with a number of mental health disorders and alcoholics have a very high suicide rate. Many authors have indicated an integral link between a person's personality and the functions of the prefrontal cortex (White and Swartzwelder, 2005; DeYoung *et al.,* 2010).This brain region has been implicated in planning complex cognitive behavior, personality expression, decision making, and moderating social behaviour. (Yang and Raine, 2009). The basic activity of this brain region is considered to be orchestration of thoughts and actions in accordance with internal goals (Miller *et al*, 2002).

Palm wine is mostly being tapped and commonly consumed in very large and chronic dimensions mostly by people in rural and sub-urban settlements. These places have virtually no functional government regulatory bodies concerned with alcohol consumption. There is paucity in literature concerning the effect of palm wine being consumed as an alcoholic beverage on the brain.

2. MATERIALS AND METHODS

Palm Wine Collection, Autentication and Extraction

Unadulterated palm wine was bought at intervals from a local palm wine tapper at Ngwo-Agu, in Udi Local Government Area of Enugu state, Nigeria. The procured palm wine were authenticated as genuine *Elaeis guineensis* Sap at the department of Plant Science and Technology, University of Nigeria, Nsukka. Thereafter, the palm wine were then diluted with 50% clean tap water, and allowed to ferment at room temperature for 24 hours before being administered to the rats.

Experimental animals

Twenty five (25) healthy male wistar rats with an average weight of 180g were procured from animal house facility of the University of Nigeria, Enugu campus. However, this study was carried out in the Animal facility of the Enugu State University of Science and Technology College of Medicine, Parklane, Enugu. The animals were kept in well ventilated breeding rooms and housed in netted iron cages**.** There were provided easy access to food (normal rat chow) and tap water *ad libitum* and were also allowed to acclimatize for 2 weeks. Ethical approval was gotten from the university's ethical clearance committee with the ethical right permission number: ESUCOM/FBMS/ETR/17/001.

Experimental Designs

The experimental procedure for this study lasted for 14 days. The experimental animals were divided into five groups (n=5) with average weight of 200g; Group A: Normal controls (n=5) were fed with normal rat chow and distilled water daily for fourteen days.

Group 2 to 5: The four experimental groups (n=5; Groups B, C, D, and E) were administered increasing volumes of palm wine via oral cannulas in progression of 1ml, 2ml, 4ml, and 8ml respectively, daily for 14 days (Oyedeji *et al.*, 2012).

Animal Sacrifice and Tissue Removal

24 hours after their last administration (Day 15), the rats were sacrificed under ketamine (100mg/ml) as anesthesia. Their respective brain cavity was opened-up and the brain carefully harvested, washed and then fixed in Bouin's fluid inside properly tagged containers for 12 hours prior to the isolation of the prefrontal cortices for histological analysis.

Histological Study

The fixed tissues were processed using the standard protocols for histological tissue processing and stained with Cresyl Fast Violet and also Hematoxylin and Eosin. The various slides of the brain tissues prepared were carefully studied under

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low and high magnification of the light microscope. Photomicrographs were taken using Amscope 14MP USB 3.0 digital microscope camera at x150 and x600 magnification respectively.

3. RESULTS

Histological Findings

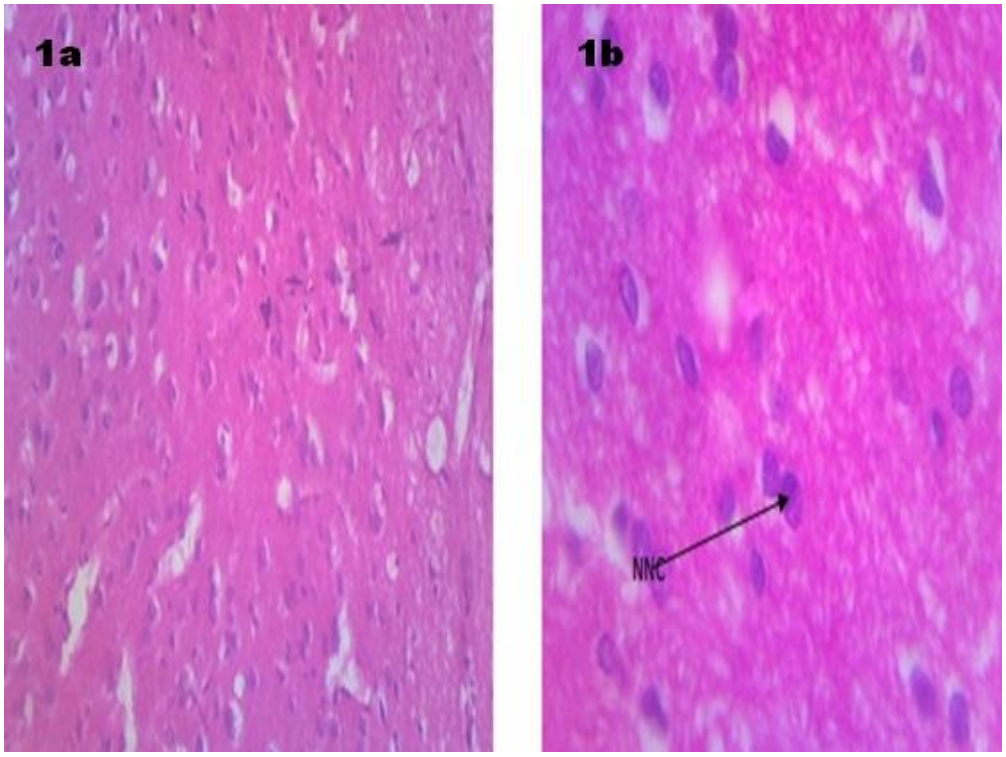

Figure 1

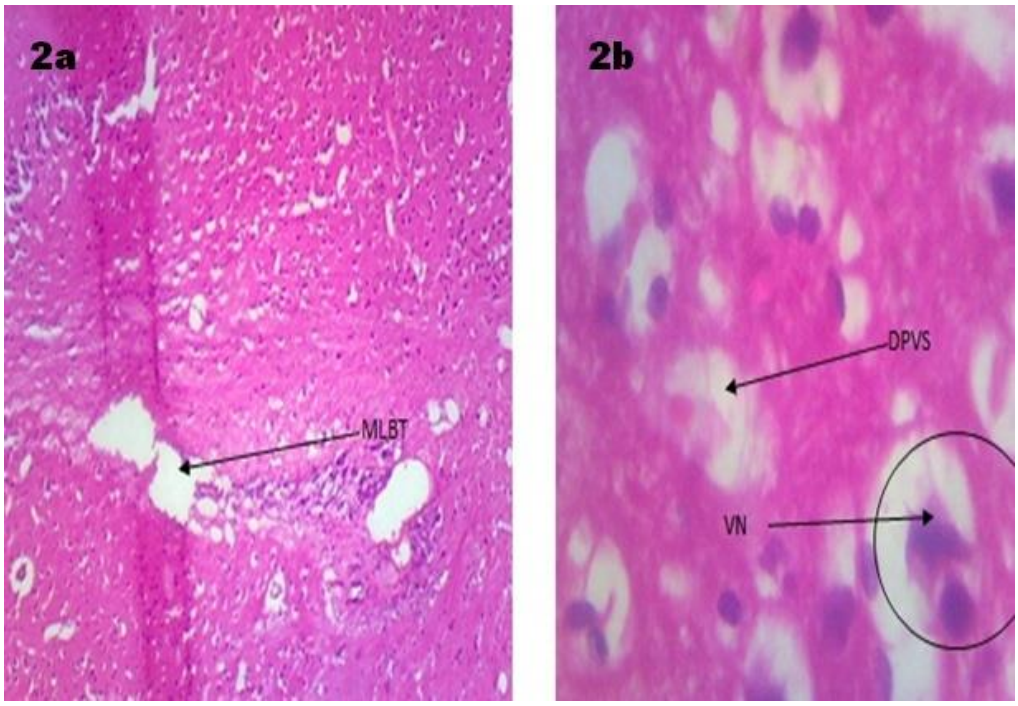

Figure 2

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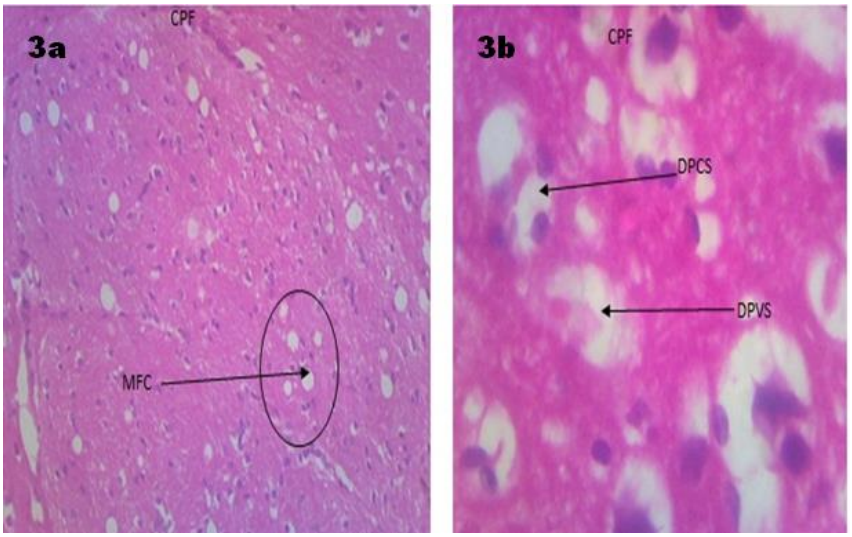

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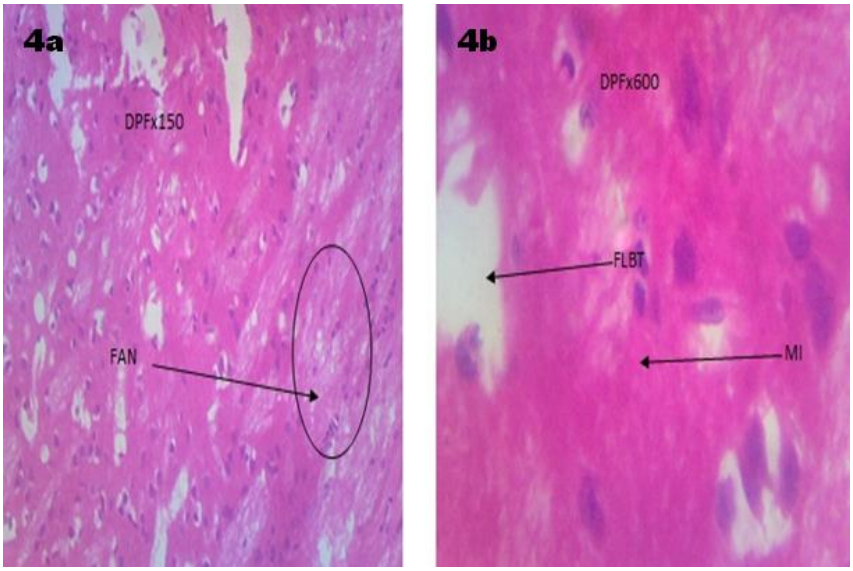

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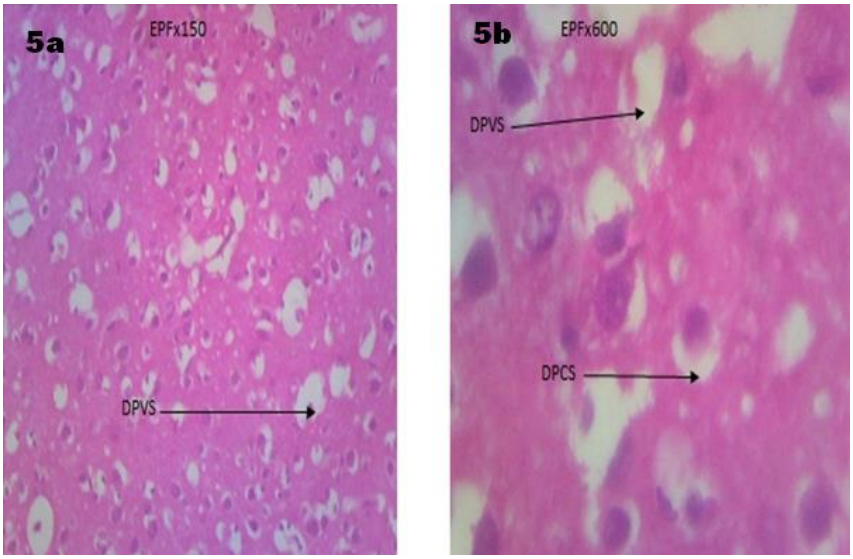

Figure 5

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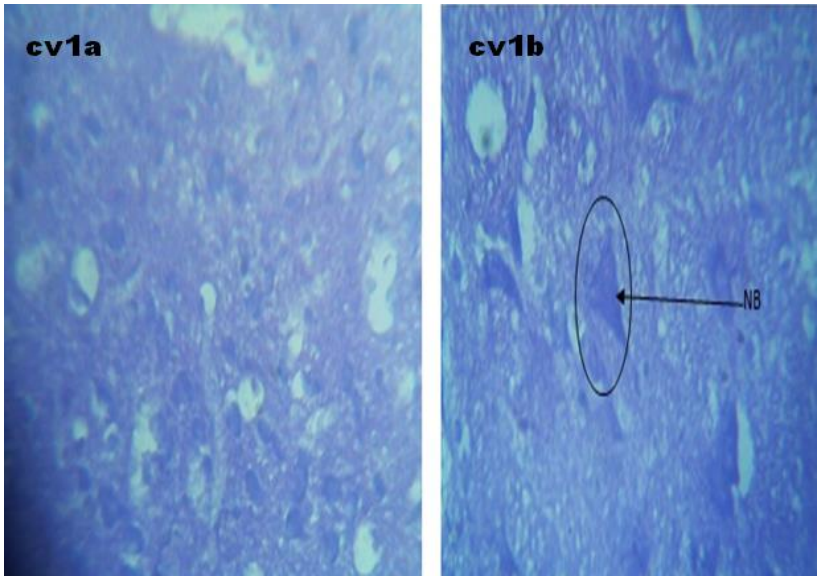

Figure 6

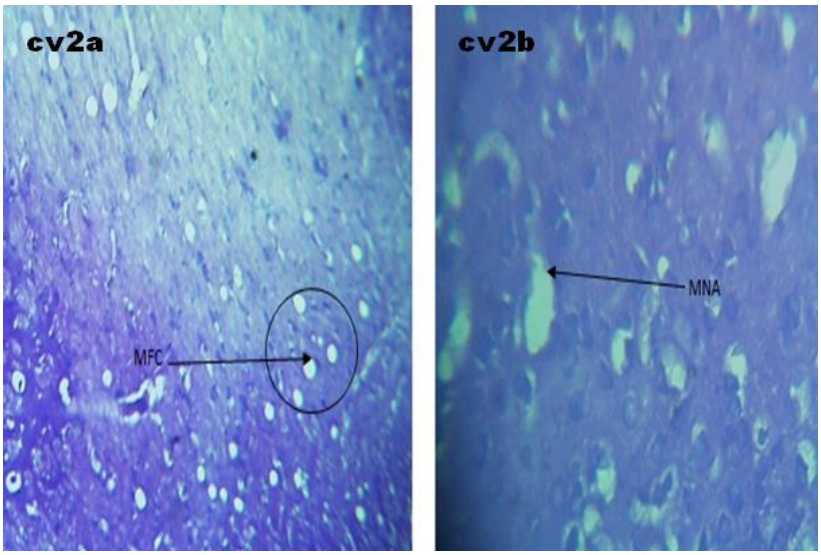

Figure 7

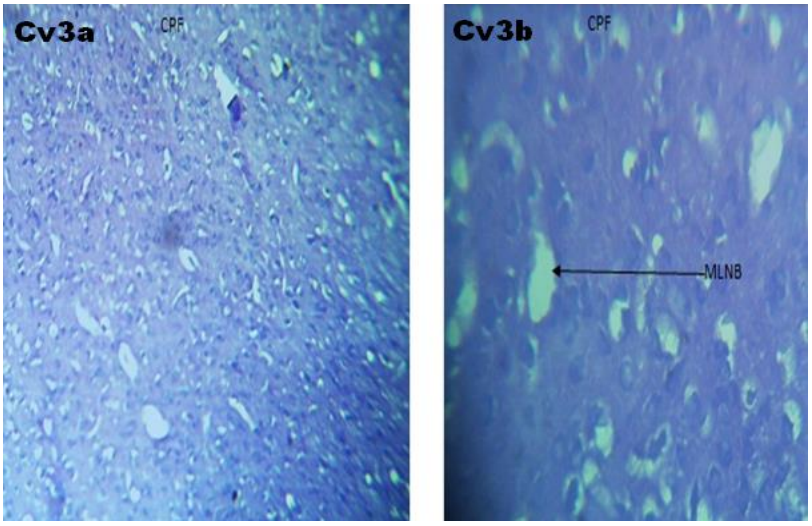

Figure 8

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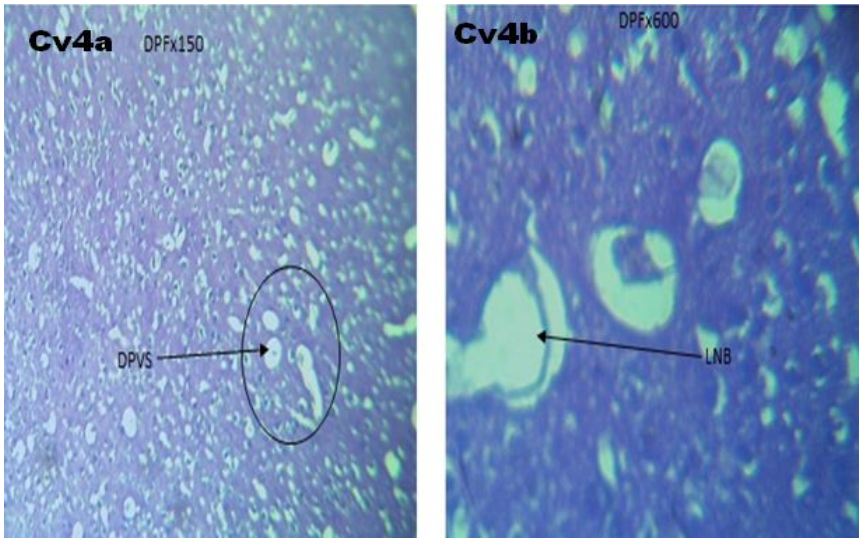

Figure 9

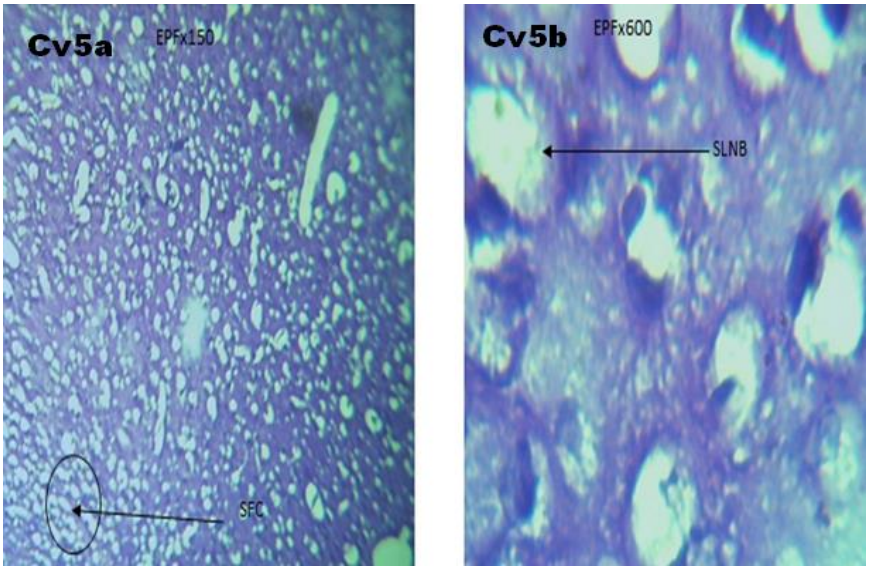

Figure 10

Figure 1: Control animal group showing Normal Neuronal Cells (NNC). H&E. A (x150) B (x600). **Figure 2**: 1ml of palm wine treated group showing moderate loss of brain tissue (MLBT), Vacuolation of neurones (VN) and dilation of perivascular spaces (DPVS). H&E. A (x150), B (x600). **Figure 3**: 2ml of palm wine treated group showing moderate to severe injury on the brain with moderate fatty change (change (MFC, dilation of perivascular spaces (DPVS) and dilation of pericellular spaces (DPCS) (oedema). H&E. A (x150), B (x600). **Figure 4**: 4ml of palm wine treated group showing moderate to severe injury brain with focal loss brain tissue (FLBT), moderate inflammation (MI) and focal area of necrosis (FAN). H&E. A (x150), B (x600). **Figure 5**: 8ml of palm wine treated group showing severe injury on the brain with dilation of pericellular spaces (DPCS) and dilation of perivascular spaces (DPVS) (edema). H&E. A (x150), B (x600).

Figure 6: Control animal group. Prefrontal cortex showing presence of Nissl bodies (NB). Cresyl Fast Violet. A (x150), B (x600). **Figure 7**: 1ml of palm wine treated group showing moderate injury on the brain with moderate fatty changes (MFC), moderate neuronal atrophy (MNA). Cresyl Fast Violet. A (x150), B (x600). **Figure 8**: 2ml of palm wine treated group showing moderate to severe in injury on the brain with moderate loss of nissl bodies (MLNB). Cresyl Fast Violet. A (x150), B (x600). **Figure 9**: 4ml of palm wine treated group showing moderate to severe injury on the brain with focal loss nissl bodies (FLNB) and dilation of perivascular spaces (DPVS). Cresyl Fast Violet. A (x150), B (x600). **Figure 10**: 8ml of palm wine treated group howing severe injury on the brain with severe fatty changes (SFC) and severe loss of nissl bodies (SLNB). Cresyl Fast Violet. A (x150), B (x600).

4. DISCUSSION

Beverages are the most active functional food category but there are specific concerns over their safety (Tamang *et al*., 2016). Palm wine is known to have alcoholic contents of which its Ethanol content (5%) varies as it increases during *fermentation (Bassir, 1962; Osim et al*., 1991; Nwaiwu and Chikezie, 2020), yet it is consumed large and chronic dimensions without government regulation. Excessive alcohol use has long been reported to cause structural and functional abnormalities in the brain and other organs (Courville, 1955; Cargiulo, 2007; Eluwa *et al.*, 2010).

Findings from this study displayed the dose-dependent deleterious effects of fermented palm wine on the histomorphorlogy of the prefrontal cortex. Severity of these effects increased as dosage increased.

The prefrontal cortex showed vacuolation of neurons, dilation of perivascular spaces, dilation of pericellular spaces (oedema), fatty changes, inflammation and focal areas of necrosis and loss of nissl bodies. Acute ethanol administration in humans has been shown to cause deficits in executive activities that are thought to require the prefrontal cortex. While limited data exists about the effects of palm wine, in particular, on the histomorphplogy of the prefrontal cortex, a number of related literatures have linked prefrontal cortex dysfunction to alcohol consumption. In one study, George *et al*., (2005) using a gambling task to assess prefrontal cortex function found that acute ethanol caused poorer decision making. Changes in the structural morphology and integrity of the prefrontal cortex have been observed that may underlie the cognitive deficits associated with chronic alcohol exposure. Alcohol-dependent subjects show reduced gray matter in the dorsolateral PFC (Jernigan *et al*., 1991). Chronic alcohol use in humans has been linked to deficits in executive function that depend on the prefrontal cortex. Goldstein *et al*., (2004) found that long-term use of alcohol has been more detrimental than cocaine in attention and executive functioning tasks. Decreases in medial frontal cortex glucose metabolism have been correlated with poor performance on the Wisconson Card Sorting Task in alcoholic subjects (Adams *et al*., 1993). It seems, though, that frontal glucose metabolism increases after a period of abstinence, suggesting that some behavioral consequences of chronic alcohol use may be reversible (Volkow *et al*., 2008). Also, abstinent subjects in one study demonstrated an increase in cognitive and executive functioning that was correlated with an increase in frontal glucose metabolism (Johnson-Greene *et al*., 1997). Chronic alcohol use is associated with reduced white matter volume throughout the cortex (de la Monte, 1988). The integrity of white matter in the right orbitofrontal cortex is significantly impaired in chronic alcoholics (Pfefferbaum and Sullivan, 2005; Harris *et al*., 2008). Indeed, recent work has demonstrated a decrease in functional heterogeneity in prefrontal cortex following ethanol administration (Volkow *et al*., 2008).

5. CONCLUSION

Upon administration, fermented palm wine (sap from *Elaeis guineensis*) was seen to bring about dose-dependent deleterious effects on the histomorphorlogy of the prefrontal cortex of the experimental animals used. Increased doses demonstrated increased severity.

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