

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

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**Abstract:** Background: Alcoholism causes both neuropsychological and neuropathological harmful modifications in the brain. Despite the alcoholic content of palm wine (sap from *Elaeis guineensis*), it is still largely consumed; most times, to chronic levels. Aim: This study seeks to investigate the effect of the sub-acute administration of fermented *Elaeis guineensis* sap (palm wine) on the hippocampus of the brain using adult wistar rats. Methodology: 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 (average weight of 200g) were divided into five groups (n=5). Group A served as the normal controls and received only distilled water daily. Palm wine treated groups 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. The rats were sacrificed under ketamine (100mg/ml) as anesthesia 24 hours after their last treatment. Their hippocampi were isolated from the carefully harvested brain tissues for histological analysis and stained with Cresyl Fast Violet and also Hematoxylin and Eosin. Results: Relative to the control groups which showed a normal histology, palm wine treated groups showed progressive dose-related injuries evidenced by neuronal/ nissl atrophies, fatty changes, vacuolation of neurons, distortion of hippocampal layers and focal areas of necrosis. The extent of damage was seen to increase as the animals received increased doses of the palm wine solution. Conclusions: Fermented palm wine administration displayed dose-dependent injurious effects on the histology of the hippocampus.

**Keywords:** *Elaeis guineensis*, Alcoholism, Hippocampus.

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## 1. INTRODUCTION

Palm wine is an alcoholic beverage with a milky appearance consumed among Nigerians majorly the Igbos of the South - Eastern part (Eluwa *et al.*, 2010). It is the sap from a distinctive specie of palm tree, called the oil palm (*Elaeis guineensis*) (Rundel *et al.*, 2002) and plays important societal roles which supports its use and consumption.

The drink is rich in nutrient and contains sugar, protein, amino acid, alcohol, vitamins and minerals (Ezeagu *et al.*, 2003). However, upon fermentation, its acceptability decreases due to the fact that the microbes responsible for fermentation

convert the sugar to alcohol, and subsequently to acetic acid leading to its loss of sweetness (Odufa, 1998). Its alcoholic content varies (Ethanol content up to 5%) but increases during fermentation (Bassir, 1962; Osim *et al.*, 1991; Nwaiwu and Chikezie, 2020). Despite its alcoholic content, palm wine is largely consumed; most times, to chronic levels.

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). Alcoholism leads to both neuropsychological and neuropathological harmful modifications which could be noticeable as a reduction in the anterograde memory (Wilkinson and Carlen, 1980; Franceschi *et al.*, 1984; Parsons, 1987), poor capacity for concentration and abstraction (Muuronen *et al.*, 1984; Carlen and Wilkinson, 1987)

The hippocampus belongs to the limbic system and plays important role in the consolidation of information from short-term memory to long-term memory and spatial navigation (Write, 1997). Studies with animals have shown that an intact hippocampus is required for initial learning and long-term retention of some spatial memory tasks, in particular ones that require finding the way to a hidden goal (Nadel *et al.*, 1975; Chiu *et al.*, 2004; Morris *et al.*, 1982; Sutherland *et al.*, 1982). Brain imaging shows that people have more active hippocampi when correctly navigating, as tested in a computer-simulated "virtual" navigation task (Clark *et al.*, 2005). A study by Maguire, *et al.* (2000) Showed that part of the hippocampus is larger in taxi drivers than in the general public, and that more experienced drivers have bigger hippocampi.

## 2. MATERIALS AND METHODS

### Palm Wine Collection, Authentication 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 was 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 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. They were provided easy access to food (normal rat chow) and tap water *ad libitum* and were also allowed to acclimatize for 2 weeks under standard laboratory conditions. 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 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. 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 (Oyedemi *et al.*, 2012). The experimental procedure for this study lasted for 14 days.

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### Animal Sacrifice and Tissue Removal

24 hours after their last administration, the rats were sacrificed under ketamine (100mg/ml) as anesthesia. Their respective brains were carefully harvested, washed and then fixed in Bouin's fluid inside properly tagged containers for 12 hours prior to the isolation of the hippocampus 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 low and high magnification of the light microscope. Photomicrographs were taken at x150 and x600 magnification respectively.

### 3. RESULTS

#### Histological Findings

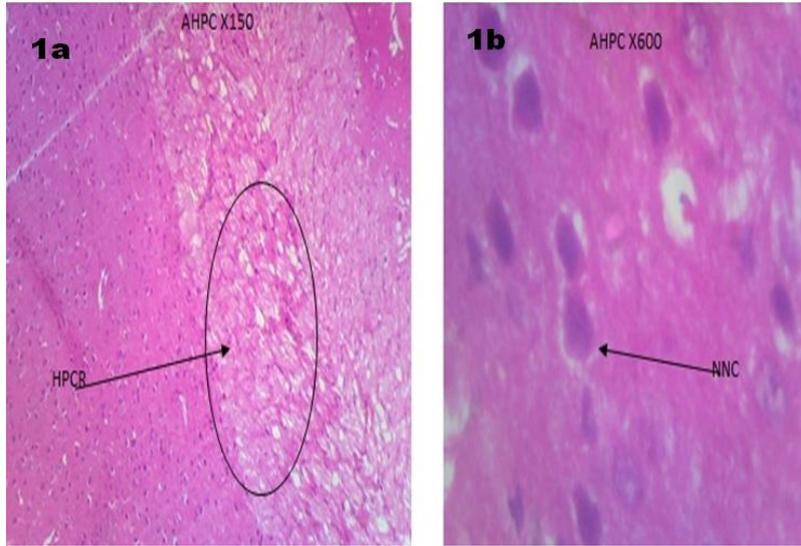


Figure 1

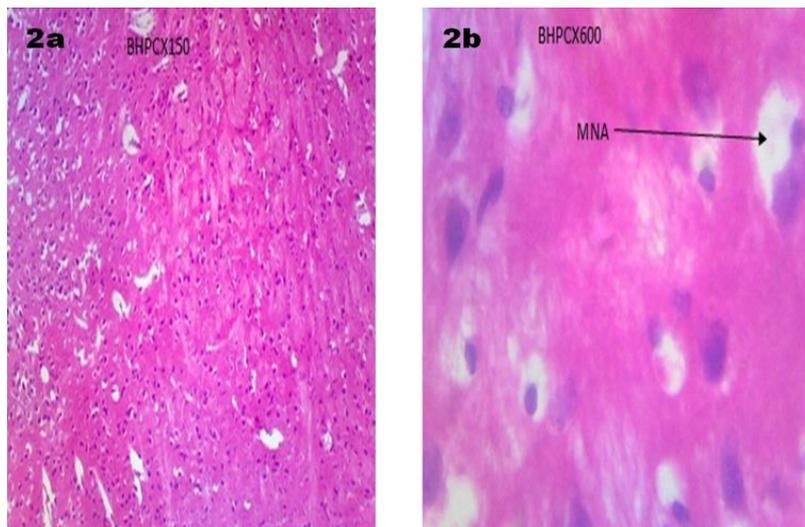


Figure 2

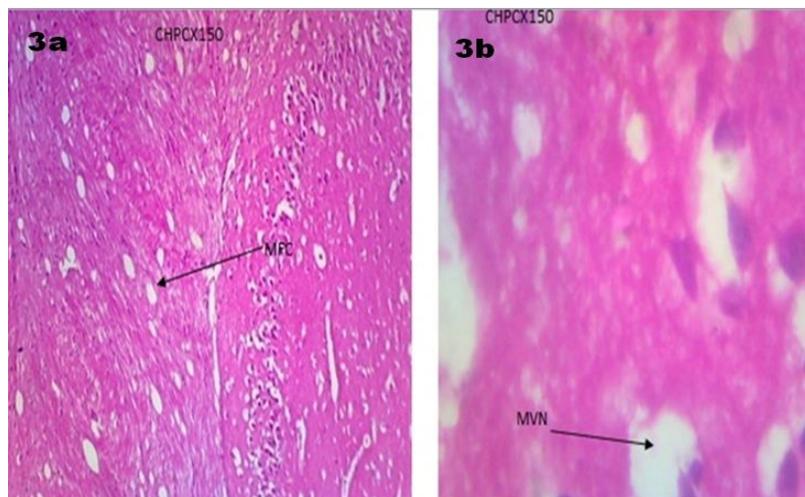


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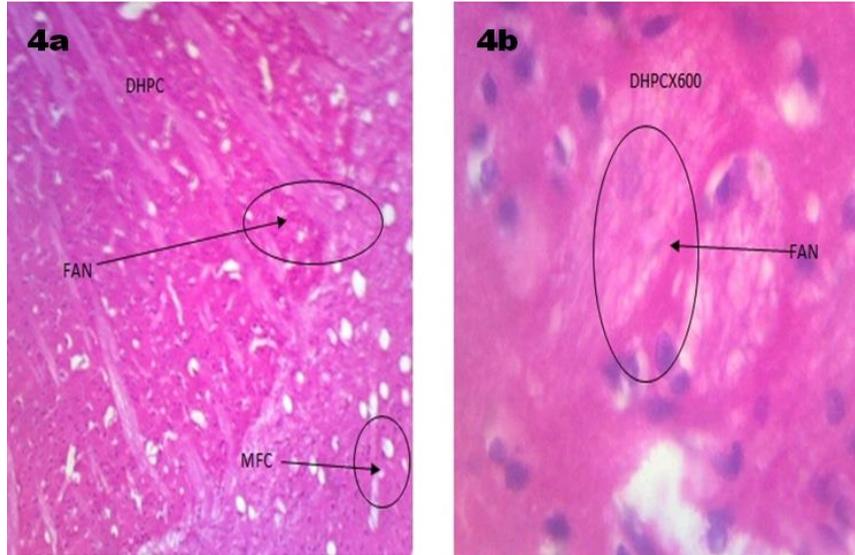


Figure 4

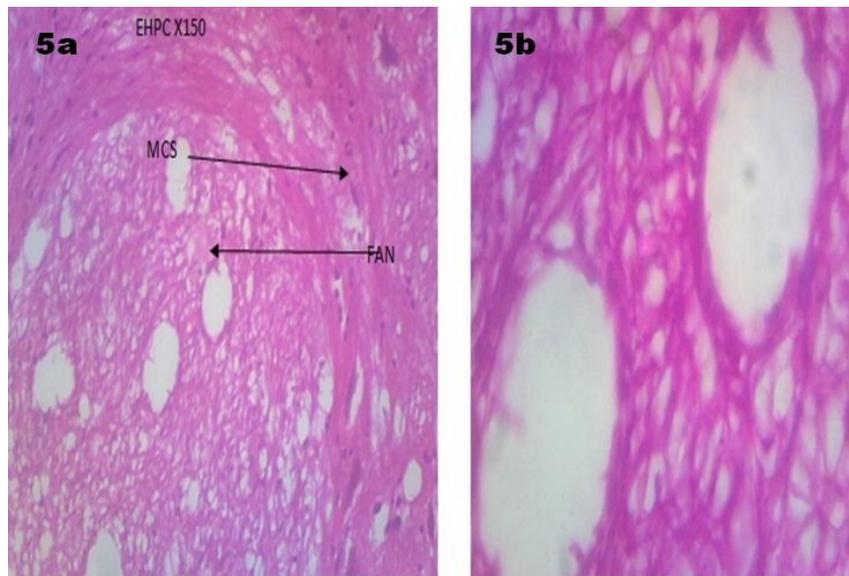


Figure 5

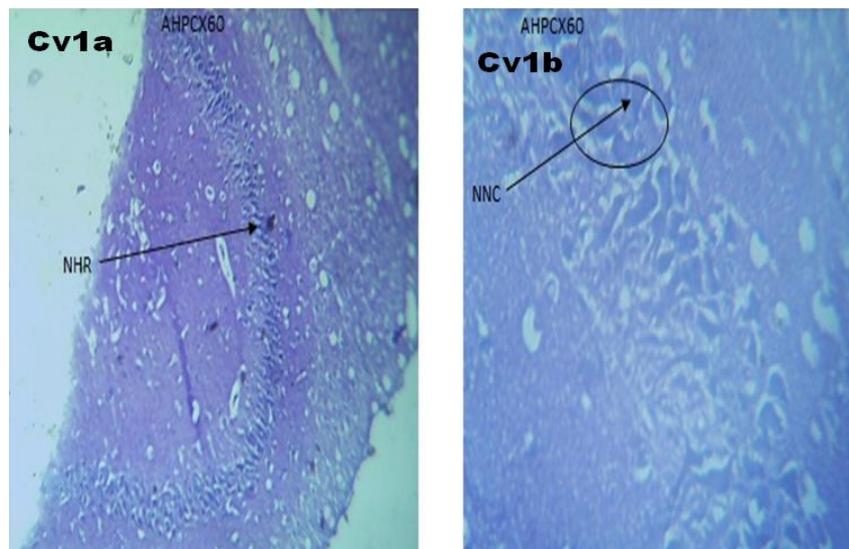


Figure 6

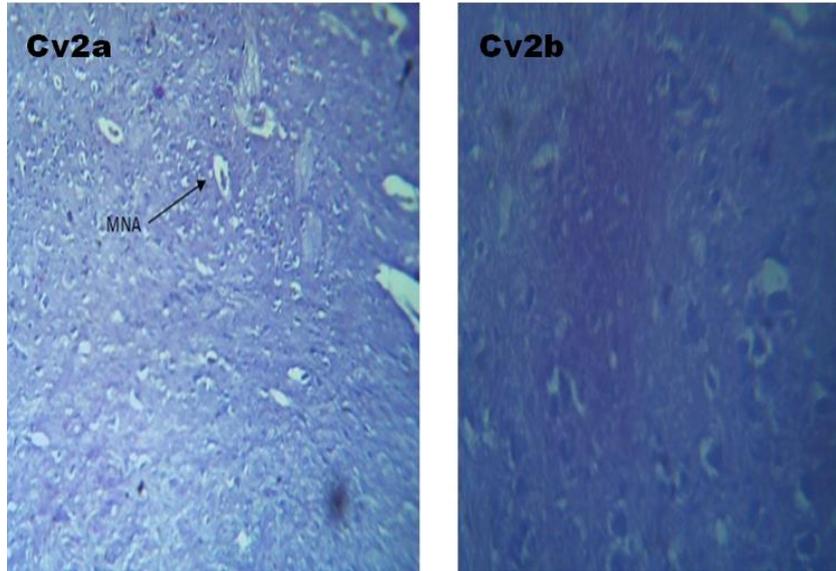


Figure 7

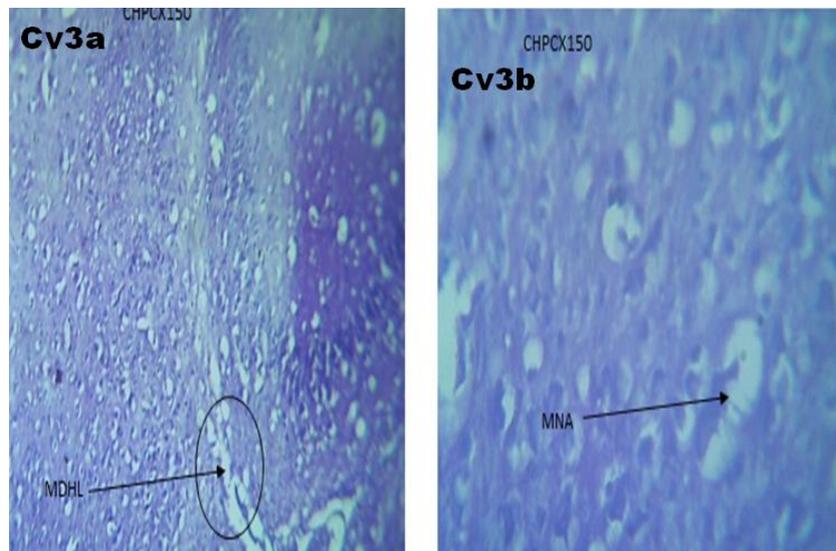


Figure 8

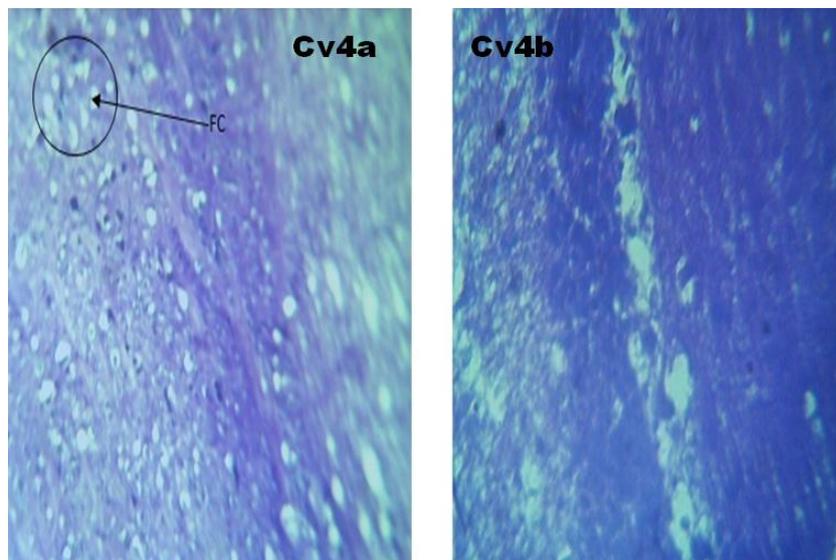
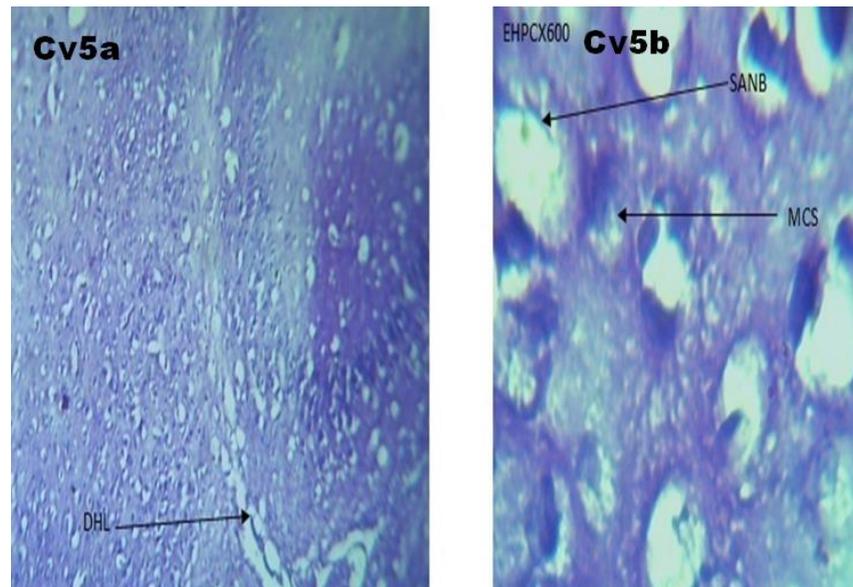


Figure 9



**Figure 10**

**Figure 1:** Photomicrographs of the hippocampus of the control animal group showing the normal hippocampus with normal neuronal cells (NNC) and hippocampal region (HPCR). H&E. A (x150), B (x600). **Figure 2:** 1ml of palm wine treatment showing mild to moderate injury on the brain with mild neuronal atrophy (MNA). H&E. A (x150), B (x600). **Figure 3:** 2ml of palm wine treatment showing moderate injury on the brain with moderate fatty changes (MFC) and moderate vacuolation of neurones (MVN). H&E. A (x150), B (x600). **Figure 4:** 4ml of palm wine treatment showing moderate injury on the brain with fatty changes (MFC) and focal areas of necrosis (FAN). H&E. A (x150), B (x600). **Figure 5:** 8ml of palm wine treatment showing severe injury on the brain with microcytic spaces (MCS) and focal areas of necrosis (FAN). H&E. A (x150), B (x600).

**Figure 6:** Photomicrographs of hippocampus of the control animal group showing the normal hippocampus with normal neuronal cells (NNC) and normal hippocampal region (NHR). Cresyl Fast Violet. A (x150), B (x600). **Figure 7:** 1ml of palm wine treatment showing mild to moderate injury on the brain with mild nissl atrophy (MNA). Cresyl Fast Violet. A (x150), B (x600). **Figure 8:** 2ml of palm wine treatment showing moderate injury on the brain with mild distortion of hippocampal layer (MDHL) and moderate nissl atrophy (MNA). Cresyl Fast Violet. A (x150), B (x600). **Figure 9:** 4ml of palm wine treatment showing moderate injury on the brain with fatty changes (FC) and absence of nissl body. Cresyl Fast Violet. A (x150), B (x600). **Figure 10:** 8ml of palm wine treatment showing severe injury on the brain with distortion of hippocampal layer (DHL), microcytic spaces (MCS), with several atrophy of nissil body. Cresyl Fast Violet. A (x150), B (x600).

#### 4. DISCUSSION

Alcoholism is being reported to cause neuropathological harmful modifications in the brain and also structural and functional abnormalities in other organs (Courville, 1955; Cargiulo, 2007; Eluwa *et al.*, 2010). Despite the alcoholic content of oil palm (*Elaeis guineensis*) sap (palm wine), it is still largely consumed to chronic levels (Nwaiwu and Chikezie, 2020).

This study identified the dose-dependent injurious consequences of fermented palm wine administration on the histology of the hippocampus using adult wistar rats. The extent of damage was seen to increase as the animals received increased doses of the palm wine solution.

The hippocampi of the control group showed normal neuronal cells and hippocampal regions. In contrast, palm wine treated groups showed progressive dose-related injuries evidenced by neuronal/ nissl atrophies, fatty changes, vacuolation of neurons, distortion of hippocampal layers focal areas of necrosis, microcystic spaces, focal areas of necrosis, distortion of hippocampal layer. These correlate with the previous works that showed the damaging effects of alcohol on the hippocampus (Mendez *et al.*, 2001; Langen *et al.*, 2002; Rubio *et al.*, 2009; Best and White, 1999; Zola-Morgan *et al.*, 1986; Slawewcki *et al.*, 2001; Da e Bellis *et al.*, 2000).

In a study by Ryabinin *et al*, 1997A, it was noted that at higher doses, alcohol significantly inhibits neuronal activity in the CA1 and CA3 pyramidal cell layers of the hippocampus. Best and White, (1999) added that this disruption has been linked to dose-dependent levels of alcohol consumption.

## 5. CONCLUSION

Fermented palm wine administration demonstrated a dose-dependent injurious effect on the histology of the hippocampus using adult wistar rats. The extent of damage was seen to increase as the animals received increased doses of the palm wine solution.

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