

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

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Abstract: Background: Local storage and preservative practices of tapped oil palm (*Elaeis guineensis*) sap notably called “palm wine” do not prevent its fermentation and thus increase its alcoholic contents. Yet, palm wine is still chronically consumed even to toxic dimensions. Aim: This study aims at evaluating the effects of the sub-acute administration of fermented *Elaeis guineensis* Sap (palm wine) on the cerebellum of the brain using adult wistar rats. Methodology: Freshly tapped 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 = 200g) were divided into five groups (n=5). Group A served as the normal controls and received only distilled water daily. Groups 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. Each brain masse was carefully harvested, and each cerebellum was isolated for histological analysis and stained with Cresyl Fast Violet and also Hematoxylin and Eosin. Results: Treated groups showed progressive dose-related injuries evidenced by fatty change, intracytoplasmic inclusions, vacuolation of purkinje cells, focal areas of necrosis and focal areas of microcystic spaces relative to the untreated control group. Conclusion: Treatment with increasing doses of fermented *Elaeis guineensis* Sap (palm wine) demonstrated clear dose-dependent deleterious effectson the histology of the cerebellum of adult wistar rats.

Keywords: *Elaeis guineensis*, Fermentation, Alcohol, Cerebellum.

1. INTRODUCTION

In parts of Africa, the inflorescences of oil palm (*Elaeis guineensis*) are skillfully injured and are tapped for sap (Onuche *et al.*, 2012). The resultant sap is called “palm wine”. Palm wine is a trending traditional alcoholic beverage commonly consumed in many social ceremonies among Nigerians including the Igbos of the South-Eastern region (Eluwa *et al.*, 2010). The drink is a rich nutrient medium that contains sugar, protein, amino acid, alcohol, vitamins, minerals and a dense population of yeast (Bassir and Maduagwu, 1978; Ezeagu and Fatunso, 2003). Fresh harvested unfermented sap is a clear and almost colorless liquid with a sweet taste and no alcohol content but soon becomes milky and increasingly less sugary and more intoxicating upon fermentation (Okoye, 2001).

Fermentation practically causes the pH to falls to 4.0 and increases its alcoholic content (Bassir, 1962). Osim et al., (1991) reported that oil palm (*Elaeis guineensis*) sap may contain up to 5% ethanol. There is a lack of proper preservative practice in rural and sub-urban settlements where there is maximum palm wine consumption. Therefore, fermentation is deemed to occur when kept for long periods. However, even upon fermentation, it is still chronically consumed even to toxic dimensions. Currently, there is paucity in the effects of fermented palm wine on the different parts of the body especially when emphasis is laid on its effect on the brain or its parts.

One of the most extensively familiar signs of alcohol intoxication is staggering gait (Sullivan *et al.*, 1995). This lack of coordination is connected to the cerebellum of the brain. The cerebellum (Latin for "little brain") is a region of the brain that plays an important role in motor control; It may also be involved in some cognitive functions such as attention and language, and in regulating fear and pleasure responses, but its movement-related functions are the most solidly established; it does not initiate movement, but contributes to coordination, precision, and accurate timing (Wolf *et al.*, 2009). It receives inputs from sensory systems of the spinal cord and from other parts of the brain, and integrates these inputs to fine-tune motor activities (Fine *et al.*, 2002). Cerebellar damage produces disorders in fine movement, equilibrium, posture, and motor learning (Fine *et al.*, 2002).

2. MATERIALS AND METHODS

Palm Wine Collection, Authentication and Extraction

Freshly tapped palm wine was bought at intervals from a local palm wine tapper at Ngwo-Agu, in Udi Local Government Area of Enugu state, Nigeria. It 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 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. 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 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. Group 2 to 5: The palm wine treated groups (n=5; Groups B, C, D, and E) were administered increasing volumes of palm wine via Oral Gavage tubes in progression of 1ml, 2ml, 4ml, and 8ml respectively, daily (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 brains were carefully harvested and the respective cerebellum isolated, washed and then fixed in Bouin's fluid for 24 hours 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 using Amscope 14MP USB 3.0 digital microscope camera at x150 and x600 magnification respectively.

3. RESULTS

Histological Findings

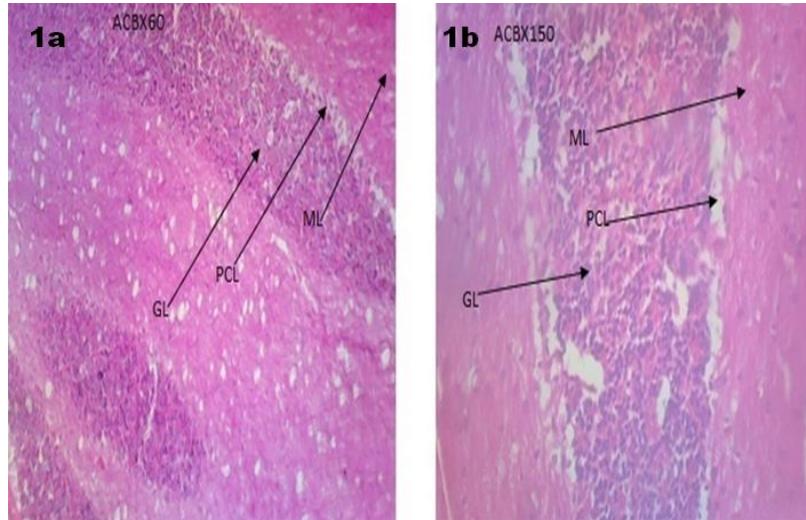


Figure 1

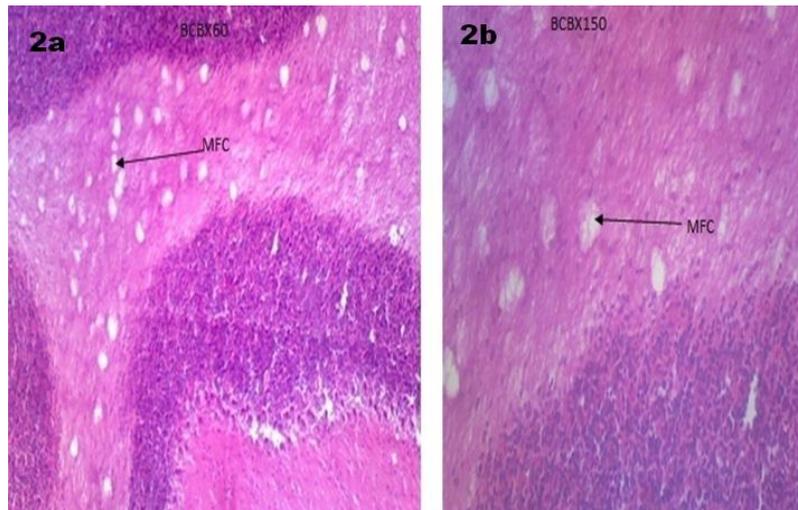


Figure 2

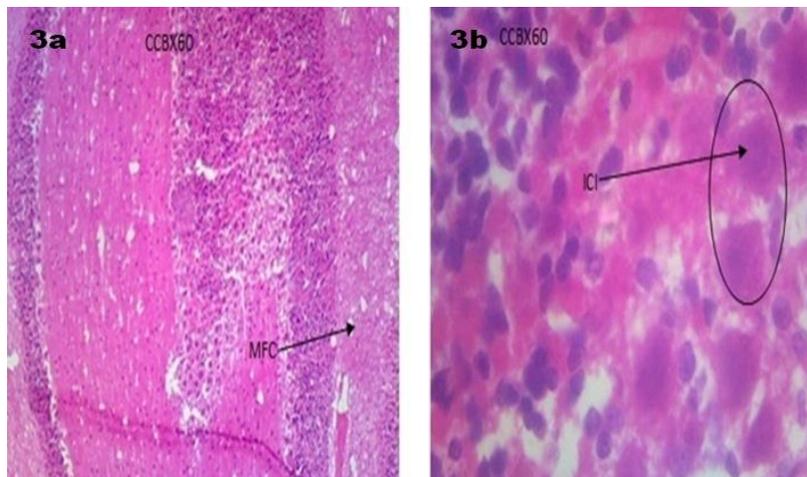


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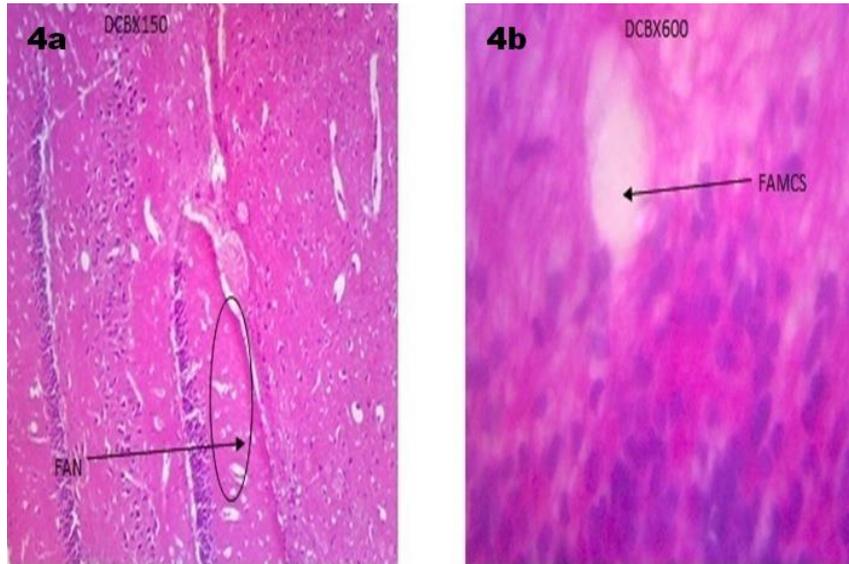


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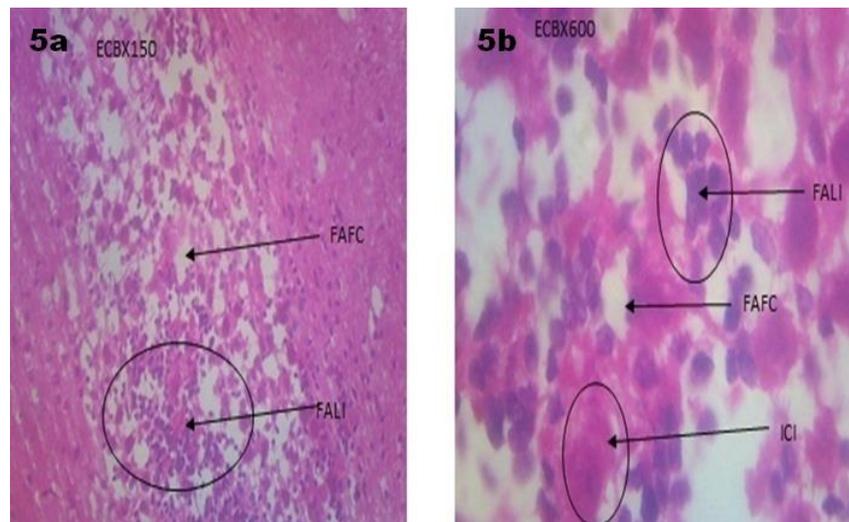


Figure 5

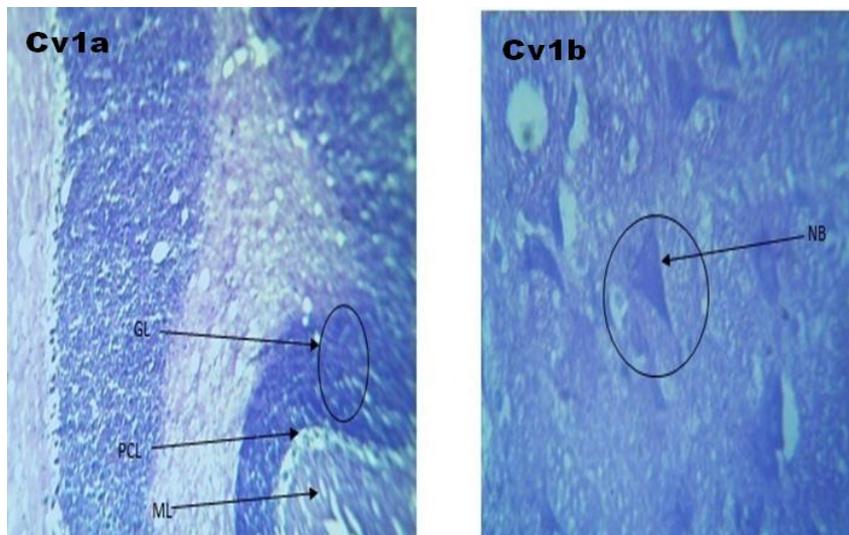


Figure 6

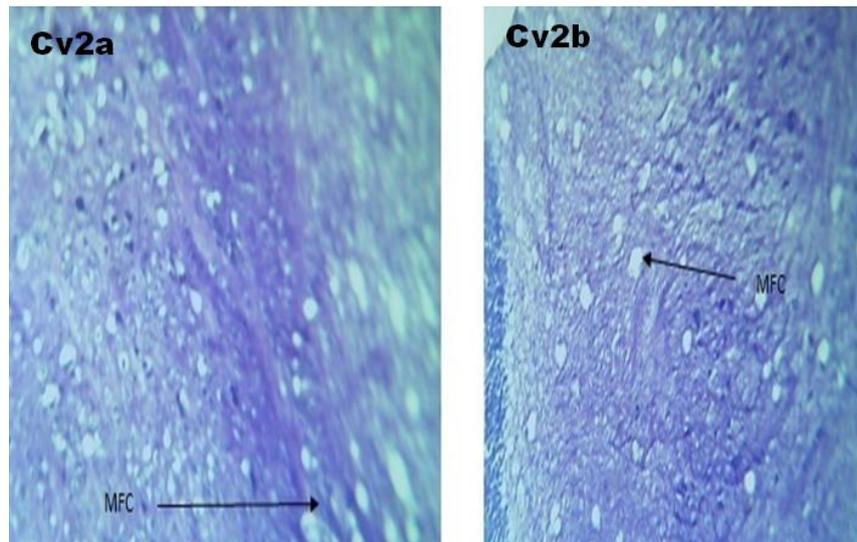


Figure 7

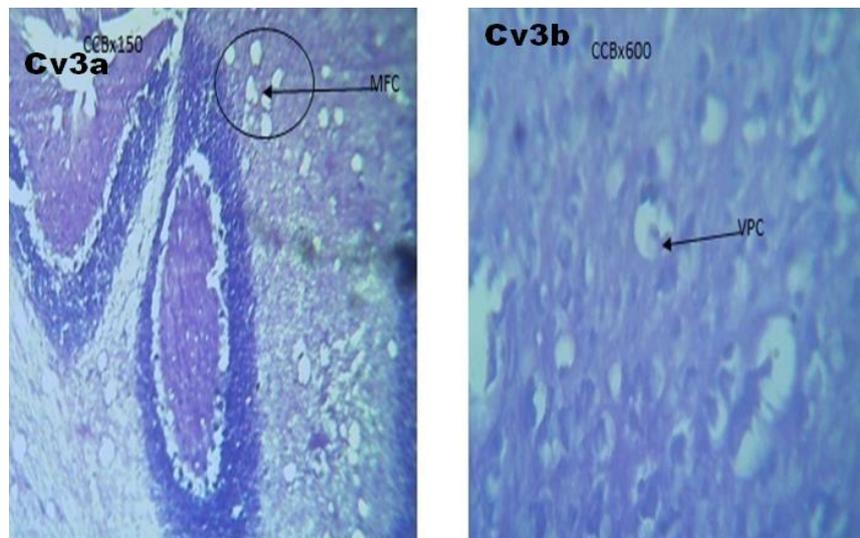


Figure 8

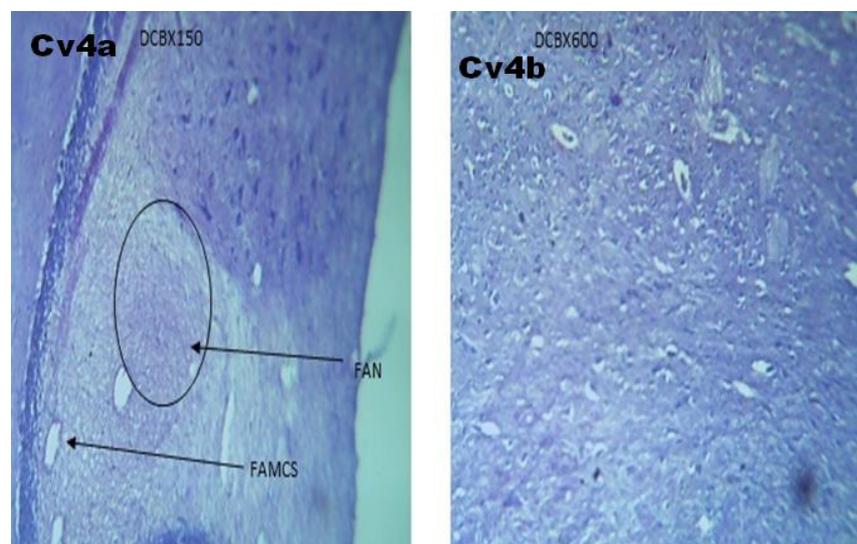


Figure 9

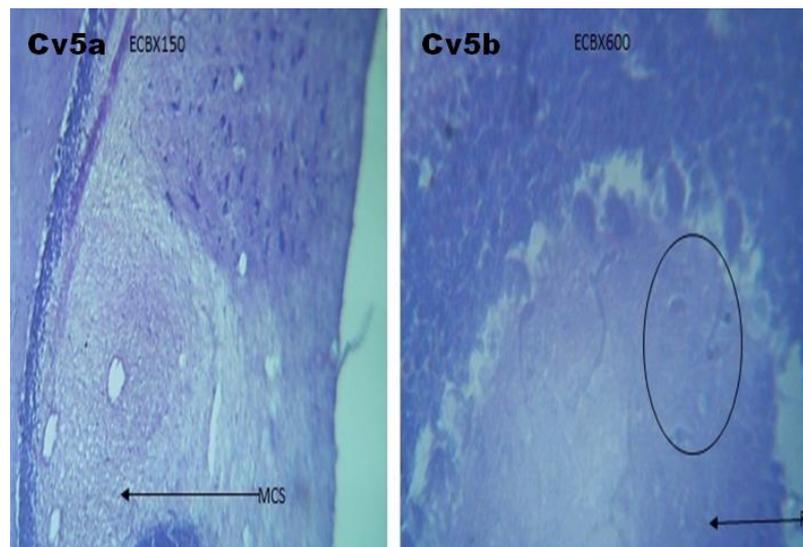


Figure 10

Figure 1: Photomicrographs of cerebellum of control rat group showing normal cerebellar cortex with different layers, outer molecular layer (ML), inner granular layer (GL), and Purkinje cell layer (PCL) in-between. H&E. A (x150), B (x600). **Figure 2:** Animal group treated with 1ml of palm wine; showing mild to moderate injury on the brain with moderate fatty changes (MFC). H&E. A (x150), B (x600). **Figure 3:** Animal group treated with 2ml of palm wine; showing moderate injury on the brain with mild fatty change (MFC) and intracytoplasmic inclusions (ICL) resembling red blood cells. H&E. A (x150), B (x600). **Figure 4:** Animal group treated with 4ml of palm wine; showing moderate injury on the brain with focal areas of necrosis (FAN) and focal areas of microcystic space (FAMCS). H&E. A (x150), B (x600). **Figure 5:** Animal group treated with 8ml of palm wine; showing severe injury on the brain with focal areas of lymphatic infiltration (FALI), intracytoplasmic inclusions (ICL) and focal areas of fatty changes (FAFC). H&E. A (x150), B (x600).

Figure 6: Cerebellum of control rats showing normal cerebellar cortex with different layers, outer molecular layer (ML), inner granular layer (GL), Purkinje cell layer (PCL) in-between, and nissl body (NB). Cresyl Fast Violet. A (x150), B (x600). **Figure 7:** Animal group treated with 1ml of palm wine; showing mild to moderate injury on the brain with mild fatty changes (MFC). Cresyl Fast Violet. A (x150), B (x600). **Figure 8:** Animal group treated with 2ml of palm wine; showing moderate injury on the brain with moderate fatty changes (MFC) and vacuolation of purkinje cells (VPC). Cresyl Fast Violet. A (x150), B (x600). **Figure 9:** Animal group treated with 4ml of palm wine; showing moderate injury on the brain with focal areas of necrosis (FAN) and focal areas of microcystic spaces (FAMCS). Cresyl Fast Violet. A (x150), B (x600). **Figure 10:** Animal group treated with 8ml of palm wine; showing severe injury on the brain with focal areas of necrosis (FA) and microcystic spaces (MCS). Cresyl Fast Violet. A (x150), B (x600).

4. DISCUSSION

Tapping oil palm sap (*Elaeis guineensis*) is a popular practice in South-Eastern Nigeria. The product called “palm wine” is a popular traditional alcoholic beverage consumed for both cultural and ceremonial motives among the Igbos of Nigeria (Eluwa *et al.*, 2010). Fermentation increases the pH and alcoholic content of palm wine (Bassir, 1962). However, it is still largely consumed even to toxic dimensions.

This study demonstrated the dose-dependent injurious effects of fermented palm wine on the histology of the cerebellum using adult wistar rats.

The cerebella of the experimental groups showed progressive dose-related injuries evidenced by fatty change, intracytoplasmic inclusions resembling red blood cells, vacuolation of purkinje cells, focal areas of necrosis, focal areas of microcystic spaces, and focal areas of lymphatic infiltration. The extent of damage noticed increased in animals that received increased doses of the palm wine solution.

These results depict a consensus with the previous works showing the deleterious effects of alcohol on the cerebellum. In one study, Torvik and Torp (1986) reported that 26.8% of alcoholics with WKS had cerebellar atrophy. Neuroimaging in vivo (Sullivan *et al.*, 2000) and examination of the cerebellum at autopsy reveal shrinkage of the anterior superior cerebellar vermis. Quantitative pathological studies have shown that there is a loss of Purkinje cells in the vermis (reduced on average by 43%) that correlates with clinical ataxia/unsteadiness (Baker *et al.*, 1999). They also noted a correlation between the loss of Purkinje cells in the lateral lobes of the cerebellum and 'mental signs'. This is particularly interesting given the recent speculation that the cerebellum is important in the organization of higher cerebral functions (Schmahmann and Sherman, 1998; Sullivan, 2003). Baker and colleagues (Baker *et al.*, 1999) showed that there is no consistent correlation between the number of neurons or the structural volume for any of the cerebellar regions in uncomplicated chronic alcoholics.

5. CONCLUSION

The administration of the fermented sap of oil palm (*Elaeis guineensis*) notably called "palm wine" established a dose-dependent injurious effects on the histology of the cerebellum of adult wistar rats. The injurious effects seen, increased with increasing doses of palm wine administration.

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