IMPACT OF LIVER DAMAGE ON THE HISTOARCHITECTURAL PROFILE OF THE CEREBELLAR CORTEX IN RATS

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ABSTRACT

Aim: This study described the histopathological profile of the cerebellar cortex and liver of rats with carbon tetrachloride (CCl_4)-induced liver damage.

Methods: Male Wistar rats weighing about 250 g were divided into 2 groups (A and B). Group A (control) rats were treated with olive oil (OO) solution only while Group B rats were treated with carbon tetrachloride (CCl_4) for 8 weeks. Thereafter, the cerebellum and liver of the rats were processed and stained with cresyl fast violet and H&E, respectively, for histopathological examinations of the cerebellar cortex and liver.

Result: Histopathological analysis shows cortical necrosis, uneven neuronal loss with varying range of vacuolations in the cerebellar cortices and moderate vascular congestion and extensive cytoplasmic damage on the hepatocytes of the CCl_4 treated rats.

Conclusion: There is of loss of hepatic function in the CCl_4 treated rats.

Key words: -Neurons, Neuropathology, Vacuolations, Necrosis, Neuronal loss

INTRODUCTION

It is becoming increasingly evident that many of the liver diseases are more than purely liver centered diseases. Animal models and clinical observations have documented that chronic liver maladies are associated with alterations and/or modification in the central nervous system (CNS) (Swain, 2006; Nguyen *et al.,* 2007; D'Mello *et al.,* 2009). Many abnormalities are highly prevalent in patients with chronic liver diseases and other diseases, and have received little attention because of a limited understanding of how these alterations within the CNS develop in the setting of peripheral organ-centered malfunctioning (Jones *et al.,* 2006; Swain, 2006; Isik *et al.,* 2007; Capuron and Miller, 2011). The causes of many complications of the CNS are conventionally thought to originate in the brain; however, due to

the metabolic dependence of the brain on peripheral organ functions and its high susceptibility to modification in systemic physiology, peripheral organ damage may contribute significantly to the neurological damage. The use of experimental and/or laboratory based models to study hepatic damage has indicated some mechanisms that may be actively involved in the development of many form of liver damage. These laboratory based models have shown the effect of these mechanisms on various organs, such as the brain, where they may trigger the onset of hepatic encephalopathy (HE) (Butterworth *et al.,* 2009). In HE, alterations in brain functions are the aftermath of a prior failure in normal liver function (Prakash and Mullen, 2010). Alteration in liver function may impede the detoxification process for toxic substances (such as ammonia) that may reach the brain and alter its function. Chronic liver injury is often associated with intense impairments in hepatocellular regeneration. According to Duguay *et al.,* (1982), cirrhosis usually occur when recurrent injury and cell loss are not adequately compensated by repair mechanisms, due to inhibition of DNA synthesis, reduced energy metabolism, insulin resistance, and oxidative stress. While changes within the brain have been largely overlooked in the setting of peripheral organ centered diseases, much of what is known of the communication pathway between the peripheral organs and the brain comes mainly from experimental studies. Elevated plasma endotoxin levels have been documented in patients with chronic liver disease (Yamamoto *et al.,* 1994; Swain, 2006). The administration of endotoxin or cytokines to healthy volunteers or animals has been shown to result into the development of deleterious histological and cytological characteristics and malfunctioning of several peripheral organs with corresponding compromising effects on the nervous system (Bluthe *et al.,* 2000; Eisenberger *et al.,* 2010). Liver cell injury is caused by variety of agents such as aspirin, carbon tetrachloride (CCl_4), paracetamol, thioacetamide, alcohol and microbes (Bilzer *et al.,* 2006; Salem *et al.,* 2010). Carbon tetrachloride (CCl_4) is an extensively studied xenobiotic that causes lipid peroxidation and toxicity (Jeon *et al.,* 2003). It is often used in the laboratory to study the mechanisms involved in

the advancement of hepatic disease and the impact of drugs on this progression in animal models (Boer *et al.,* 2009). Activation of CCl_4 by cytochrome P450 to trichloromethyl and trichloromethyl peroxy radicals is said to promote lipid peroxidation and protein oxidation in the liver, resulting in membrane damage and liver injury (Scarpelli and Iannaccone, 1990). Hepatic failure is a clinical condition that often results in multiple organ failure and death (Sheweita *et al.,* 2001; Konopacka *et al.,* 2008; Kung *et al.,* 2008). Animal tissues are constantly coping with highly reactive species, such as superoxide anion, hydroxyl radicals, hydrogen peroxide, and other radicals generated during numerous metabolic reactions (Castillo, 1992; Hartley *et al.,* 1999; Cabre *et al.,* 2000; Melin *et al.,* 2000). It is known that oxidative stress resulting from increased free radical production after CCl_4 intoxication may play a crucial role in the degenerative processes in many tissues of the body (Szymonik-Lesiuk *et al.,* 2003). The cerebellum is a motor structure involved in posture, maintenance of balance, coordination of voluntary movement and motor learning (Alexander *et al.,* 2012). Impairment of cerebellar functions often leads to impairments in motor control and posture because majority of the cerebellar outputs are a compliment of the motor system (Bolduc *et al.,* 2011). According to Peterson *et al.,* (1990), different models of chronic liver failure have shown a disruption in the functional integrity of several regions of the brain such as the cerebral cortex, hippocampus, striatum and thalamus. It has also been reported that in cases of hepatic damage, certain toxic substances may reach the brain and provoke morphological changes affecting only the astrocytes. With all of the above in mind, the purpose of our study is to evaluate the implication of liver-induced damage on the neuronal profile of the cerebellar cortex in rat.

MATERIALS AND METHODS
Animal Care
Sixteen male Wistar rats (Pharmacy Department, Obafemi Awolowo University, Ile-Ife, Nigeria), weighing about 250 g on arrival were housed in two polycarbonate cages with stainless steel lids, under a light cycle with a phase of 12 h from 7 a.m. to 7 p.m., with free access to chow and water. Two experimental groups were established: a

control group (A) treated with olive oil, and a CCl_4 treated group (B). Treatments were administered 3 times a week for 8 weeks. All experiments were carried out according to the Guide for the Care and Use of Laboratory Animals by the National Institute of Health (NIH Publication No. 8023, 1978).

Inducing Liver Damage
Hepatic damage was induced in the CCl_4 treated group according to the method of Adekomi *et al.* (2013). Briefly, a blend of 0.2 mL of CCl_4 and olive oil in a ratio of 1:1 (v/v) was administered intraperitoneally 3 times a week for 8 weeks. The choice of CCl_4 dosage was according to the dose employed in the study of Mu˜noz *et al.,* (1998).

Histopathological Study
Twenty-four hours after the administration of the last dose, the rats were sacrificed under ether anesthesia. The skulls were opened, brains were removed and the cerebellar cortices were dissected out for histopathological studies. The dissected cerebellar cortices were washed with normal saline followed by 50 ml of 4% paraformaldehyde in PBS, postfixed in neutral buffered formalin for 7 days. For cresyl violet staining, a 1:5 series of sections was mounted onto coated slides, dried, and stained in 2.5% cresyl violet acetate (Sigma, St. Louis, MO). Liver tissue was fixed overnight in 10% formol saline and embedded in paraffin. Tissue sections of 5μ thick were stained using the Leica Autostainer XL for Hematoxylin and Eosin.

Enzymes and Marker Study
The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were quantified in the serum, while the concentration of glutathione peroxidase (GP_X) and malondialdehyde (MDA) were quantified in the cerebellar and liver tissue homogenate respectively. For the assessment of these markers and enzymes, their respective quantity and/or concentration were measured with the spectrophotometer as described by Padurariu *et al.,* (2010) and Adekomi *et al.,* (2013).

Statistical Analysis
Data were evaluated statistically using Student's t-test with SPSS/14.0 software (SPSS Inc, Chicago,

USA) and Excel 2007 (Microsoft Corporation, USA) and were expressed as means ± standard error of mean (SEM). A value of $p<0.05$ was considered to indicate a significant difference between groups.

RESULTS
Histopathological Observations
Treatment with CCl_4 induced neuronal damage in the cerebellar cortex as shown with arrow heads in Plate 2B. The characteristics of cell death observed in the cerebellar cortex of the CCl_4 treated rats include neurodegenerative features of neuronal death with distorted membranes and degeneration of cytoplasm. There was loss of Nissl's bodies in the neurons of the cerebellar cortex of the rats with CCl_4-induced hepatic damage. Structural evidence from previous study suggests that a compromise in liver functions can affect the prefrontal cortex (Adekomi *et al.,* 2013). Comparing the treatment groups in Plates 2A and 2B against the control (Plates 1A and 2B), neuronal degeneration can be said to have occurred. It was observed that the administration of CCl_4 induced changes in hepatocellular profile of the treated rats and these include; moderate vascular congestion and extensive cytoplasmic damage in the hepatocytes (Plates 4A and 4B). The hepatocellular profile of the rats in the control group was well preserved (Plates 3A and 3B). To confirm that the histological alterations shown in Plates 4A and 4B represent significant hepatocellular damage, the activities of alanine aminotransferase and aspartate aminotransferase were measured in the rats examined in Plates 3A, 3B, 4A and 4B. Administration of CCl_4 produced significant $(p<0.05)$ increase in the activities of these enzymes (ALT and AST) in the treated rats compared with the control rats (Figures 5 and 6). The concentration of lipid peroxidation index, MDA in the livers and cerebellar cortices of the CCl_4-treated rats were significantly $(p<0.05)$ higher when compared with the control rats (Figure 7). Also, compared with the control rats, GP_X concentration in the livers and cerebellar cortices were significantly $(p<0.05)$ higher in the CCl_4-treated rats (Figure 7).

DISCUSSION AND CONCLUSION
Much research has been directed toward understanding the essential processes and the

mechanistic underpinnings of the impact of liver damage on the brain via the brain-liver axis. This study showed that liver damage caused by CCl_4 is capable of triggering a deleterious cascade of biochemical and physical events in the rat cerebellar cortex. It has been suggested that hepatic damage decreases the expression of both GLT-1 protein and its mRNA. It also increases the extracellular concentration of Glu, which may over activate NMDA receptors, increase calcium influx into the postsynaptic neurons, and cause neuronal damage (Knecht *et al.,* 1997; Michalak and Butterworth, 1997). Although CCl_4 is primarily metabolized in liver, its determinant effect on the brain is well documented (Boer *et al.,* 2009). The lipid solubility of CCl_4 allows it to cross cell membranes and be deposited in different tissues such as the liver, brain and testes (Szymonik-Lesiuk *et al.,* 2003). Free radicals have been implicated in multiple CNS disorders (Kuloglu *et al.,* 2002) because the CNS tissue is highly sensitive to oxidative stress due to its high oxygen consumption, high iron and lipid contents, especially polyunsaturated fatty acids, and the low activity of antioxidant defenses (Carbonell and Rama, 2007). While the effect of CCl_4 on the liver has been extensively studied, the possible damage to several of the brain regions and the brain as a whole has received less attention. There was considerable venous congestion in the liver (Plates 4A and B). This congestion as suggested by Hong *et al.,* (1991) and Wijetunga *et al.,* (2003) is a typical early marker of hepatotoxicity. It could also be an evidence of posthepatic vascular constriction (Chen, 2007; Wang *et al.,* 1990), and/or direct drug-induced hepatotoxic damage to the liver which may ultimately compromise the metabolic functions of the liver. Similarly, serum AST and ALT are well established and sensitive markers for liver damage and were elevated after CCl_4 treatment. Although increases in AST or ALT could represent damage to other tissues, the elevations in both, along with the hepatic histopathological findings, support the claim that CCl_4 does cause liver damage (Charles *et al.,* 2012; Adewole *et al.,* 2007; Junnila *et al.,* 2000). The adverse effect of CCl_4 on the liver of the treated rats also include severe cytoplasmic loss (Plates 4A and B), suggestive of cellular degeneration (Rautou *et al.,* 2008). It has been suggested that compromised liver functions resulting from inborn error of metabolism or frank liver damage can contribute to compromised neurological symptoms, including altered mental status, peripheral neuropathy, and coma (Felipo and Butterworth, 2002). The current findings showed that CCl_4 produced significant elevated activities of AST and ALT matching with the compromised histopathological state observed in the liver. This corroborates with the study of Wegwu and Obi (2007). The concentration of the markers of oxidative stress were statistically consistent in both the liver and cerebellar cortex, therefore CCl_4 appeared to cause neurotoxic effect via oxidative stress. Similar result was observed in the study of Adekomi *et al.,* (2013). Metabolic stress impairs the cellular functions of the CNS, including myelin maintenance. The extent to which liver damage related neurodegeneration occur would likely correlate positively with the level of compromise in the gastrointestinal and hepatic detoxification. In essence, CCl_4-induced hepatotoxicity with increased oxidative stress and a marked alteration in the activities of AST and ALT, would likely mediate their adverse effect on the brain. In conclusion, this study agrees with earlier report that CCl_4-induced hepatic damage causes neurotoxic effect through oxidative stress and also compromises the integrity of the neuron and neuropil in the cerebellar cortex of CCl_4 treated rats.

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Fig 5. Serum AST activities after treatment with PBS and CCl_4 respectively in the livers of the control and treated rats (* $p < 0.05$).
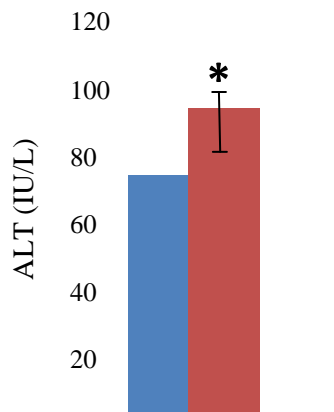
Fig 6. Serum ALT activities after treatment with PBS and CCl_4 respectively in the livers of the control and treated rats (* $p < 0.05$).
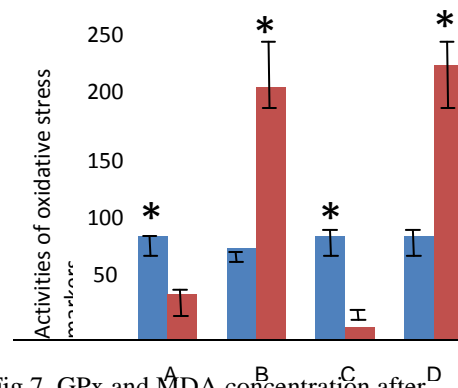
Fig 7. GPx and MDA concentration after treatment with PBS and CCl_4 respectively in the cerebellar cortex and liver of the control and treated rats (* $p < 0.05$).

Key:
A= GPx activities in the Cc
B= GPx activities in the liver
C= MDA activities in the Cc
D= MDA activities in the liver
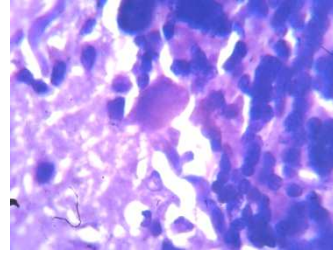Cc = Cerebellar cortex   Control   CCl4 treated

Plate 1A

Plate 1B

Plate 1; Cresyl violet-stained neurons in the cerebellar cortex showing normal neuronal profile of the neurons in the cerebellar cortex of the representative rats in the control group. The adjacent photomicrograph (B) is that of the yellow boarder region on (A) at higher magnification (A = 100x; B = 400x).
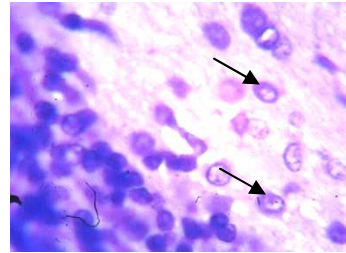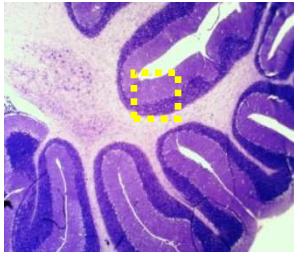

Plate 2A

Plate 2B

Plate 2; Cresyl violet-stained neurons in the cerebellum showing evidence of neuronal cell death in the cerebellar cortex of CCl$_4$ treated rats. The adjacent photomicrograph (2B) is that of the yellow boarder region on 2A at higher magnification. The arrows depict neuronal cell bodies with fragmented cytoplasm and nucleus in the molecular and granular cell layer of the cerebellar cortex respectively (2A = 100x ; 2B = 400x).
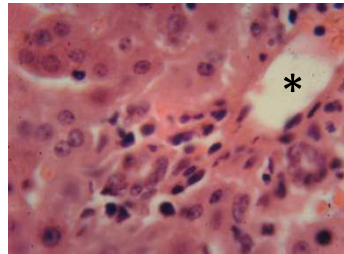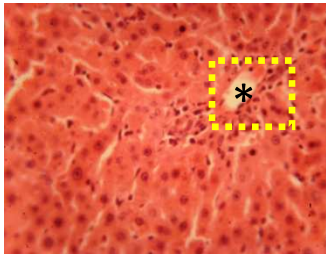

Plate 3A

Plate 3B

Plate 3; Hematoxylin and eosin stained section of the liver of the representative rats in the control group with preserved and well defined hepatic profile (magnification, 1A = 100x ; 1B = 400x).
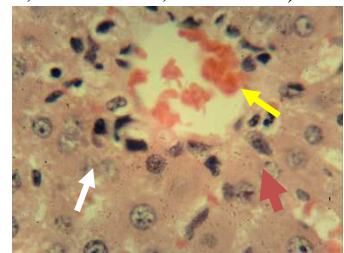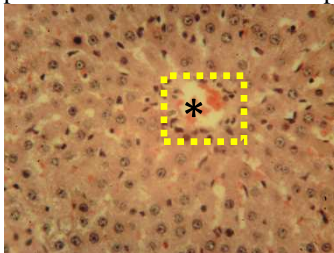

Plate 4A

Plate 4B

Plate 4; CCl$_4$ caused hepatocellular damage. Hematoxylin and eosin stain was used to examine the effect of CCl$_4$ on the liver of the CCl$_4$ treated rats. A representative image of a hepatic lobule near central vein is shown (asteriks denote central vein). The image in this figure is the representative of all the rats in the CCl$_4$ treated group. Evident in this slide is moderate vascular congestion (green arrow head). This congestion may be responsible for the extensive cytoplasmic disappearance in the hepatocytes of the CCl$_4$ treated rats (red arrow). Also, there is moderate vascular and sinusoidal congestion evident throughout the liver (white arrow) (magnification, 2A = 100x ; 2B = 400x).