ORIGINAL ARTICLE

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MICROANATOMICAL AND BIOCHEMICAL CHANGES OF THE CEREBELLUM FOLLOWING ETHANOL GAVAGE IN ADULT WISTAR RATS

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ABSTRACT

Ethanol consumption has been linked with social and medical problems, coupled with damage of multiple organs including the cerebellum. The present study is aimed at investigating the histological and biochemical changes in the cerebellum of Wistar rats associated with ethanol exposure. The experimental animals were grouped into five groups designated as Group 1 which served as the control group and was given distilled water, Groups 2,3,4 and 5were given 40%, 25%, 12% and 5% v/v of ethanol respectively. Each of the experimental animals was administered 10mls/kg body weight of the stock solution for 42days after which the animals were sacrificed humanely. The cerebellum was removed, fixed in Bouins fluid for histological study while brain homogenates were prepared and used for the biochemical studies. Data was analyzed using one-way ANOVA and Tukey HSD Post-Hoc comparison test was used to determine where the difference lies. Oxidative stress studies showed significant increase and decrease in some oxidative stress markers when compared to the control group ($p<0.05$). The sialic acid studies showed a dose dependent decrease in the mean sialic acid concentration of the cerebellum across the groups when compared to the control ($p<0.05$). The histological studies showed the following changes; necrotic Purkinje cells with reduced linear distribution of Purkinje cells, in section of the cerebellar tissue of rats in Groups 2 and 3 with sections from Groups 4 and 5 remaining relatively normal when compared to the slide from the control group. Exposure to ethanol from the present studies showed a dose dependent effect on the cerebellum, as manifested in the histological and biochemical studies.

Key words: Ethanol gavage, Histological, Biochemical changes, Cerebellum

INTRODUCTION

Alcoholism is a term used for any drinking of alcohol that could result in problems (Littrell, 2014). There are 2 billion people worldwide who consume alcoholic beverages (WHO, 2004). From earliest time to present, alcohol had been adorned with an important role in worship (Babor, 1986). Alcoholic beverages have been a source of needed nutrition, with different concentration of ethanol in different alcoholic beverages. In dilute aqueous solution, ethanol has a somewhat sweet flavor (Shakhashiri, 2009). Chronic consumption of alcohol over time have been said to be one of the major sources of social and medical problem in our society today (Moss, 2013). Fetal alcohol syndrome is the leading cause of many mental retardation in the western world (Astley et al., 2002). Ethanol's harmful effects include neuronal cell death, impaired differentiation, reduction of neuronal numbers, and weakening of neuronal plasticity (Kumar, 2013).

The cerebellum is the largest part of the hind brain, bulging back behind the pons and the medulla oblongata (Martin, 2002), involved in coordination of voluntary movement (Luo, 2015). Various invitro and invivo studies suggest that prenatal and postnatal ethanol exposure induces elevated level of oxidative stress either by generation of free radicals or disruption of antioxidative defense mechanisms, thereby, promotes apoptotic cell death in the brain of

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rodents, with the cerebellum appearing to be the most vulnerable (Olney et al., 2002; Luo, 2012). Sialic acid is a family of nine carbon acidic monosaccharide that occurs naturally at the end of sugar chains attached to the cell surface and soluble proteins (Schnaar, 2014).Studies have reported highest concentration of sialic acid in the brain where it participates as an integral part of gangliocide structure in neural transmission (Wang, 2012). Sialic acid serves the purpose of immune moderation (Traving and Schauer, 1998). It also appears to increase both memory and cognitive performance (Morgan and Winick, 1980). The present study was aimed at investigating the histological and biochemical changes in the cerebellum associated with ethanol exposure.

MATERIALS AND METHODOLOGY

Wistar rat, light microscope, absolute ethanol, test tubes, water bath, sialic acid assay reagent, tissue processing reagent, distilled water, 2ml syringe, scalp vein set, pestle and mortar, weighing balance, acetate buffer, dissecting set

Twenty-five adult Wistar rats of both sexes, weighing between 180-200 were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were kept in animal house of Human Anatomy Department for two weeks to allow them to acclimatize. They were fed with vital feed and allowed access to drinking water *ad libitum.*

The experimental animals were grouped into five with each group containing five (5) Wistar rats. Group 1 served as the control and administered distilled water only, Group 2 were administered 40% v/v of ethanol, Group 3were administered 25% v/v of ethanol, Group 4were administered 12% v/v of ethanol and Group 5were administered 5% v/v of ethanol.

Absolute ethanol from Sigma Aldrich with CAS Number: 64-17-5was obtained from a reputable company and various concentrations were prepared thus: For 40%: 40ml of absolute alcohol was diluted in 60ml of distilled water, for 25%: 25ml of absolute alcohol was diluted in 75ml of distilled water, for 12%: 12ml of absolute alcohol was diluted in 88ml of distilled water and for 5%: 5ml of absolute alcohol was diluted in 95ml of distilled water.

Each of the treatment animal received 2mls/kg body weight of ethanol from the stock solution. The administration was done orally by gastric intubation and lasted for a period of 42 days after which the animals were sacrificed humanely after using 5mg/kg intravenous thiopental sodium anaesthesia. The range for induction of anaesthesia using thiopental sodium is 3-7mg/kg (Kataria *et al.*, 2012). The cerebellar tissues were removed and fixed in Bouin's fluid for histological studies using toluedin blue. Brain tissue homogenates were prepared for the biochemical studies.

Sialic acid assay

1) *Preparation of cerebellum for sialic acid assay*

The wistar rat cerebellum were removed and homogenized in 10 mM acetate buffer (pH 5.5). The sample was centrifuged at 1000rev for 10 minutes to obtain a supernatant that was used in the assay of sialic acid.

2) *Thiobarbituric assay of sialic acid*

Sample (0.5ml) was taken into a test tube, Sodium periodate (0.25ml) was added to it and allowed to stand for 20 minute, Sodium arsenite (0.2ml) was added after which thiobarbituric acid (2.0ml) was also added, the mixture was boiled for 10 minute until a bright red color was observed, the solution was then cooled and (5ml) of butanol-HCl was added, the absorbance of the supernatant was taken at 550 nm against the blank (Warren, 1959).

Estimation of oxidative parameters

1) Determination of catalase activity: Catalase activity was determined using the method described by Sinha, (1972).
2) Determination of superoxide dismutase: Activity of Superoxide Dismutase (SOD) was determined by a method described by Fridovich, (1989).
3) Assessment of malondialdehyde: Malondialdehyde as evidenced by the formation of TBARS which was measured by the method of Niehaus and Samuelson (1968).

The trimmed cerebellar tissues were processed with the aid of a KD-TS6B, Automatic Vacuum Tissue Processor inthe Department of Human Anatomy, Ahmadu Bello University, Zaria. Sections of the processed tissues were cut using rotatory microtome at 5μ, the microtome was obtained from Department of Human Anatomy, followed by subsequent staining with toluedine blue. The photomicrograph of the slides was carried out at magnification 400 using MD900 Am scope digital camera.

The statistical analysis was done using the statistical package for social scientist (SPSS version 18). All data were expressed as mean ± SEM. The difference between means were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc tests where necessary, $p<0.05$ was considered statistically significant.

RESULTS

The result of the sialic acid studies showed a dose decrease in the mean sialic acid concentration of the cerebellum across the groups. A significant decrease in the mean concentration of both bounded and total sialic acid was observed in group 2 when compared to the control ($p<0.05$) as shown in Table 1.

Table 1: Effect of ethanol gavage on sialic acid concentration in cerebellar tissue of adult wistar rats

Groups	Treatment	Free sialic acid (mg/ml)	Bounded sialic acid (mg/ml)	Total sialic acid (mg/ml)
1	Control (distilled water)	0.18±0.02	0.37±0.02	0.56±0.04
2	40% v/v of ethanol	0.19±0.05	0.18±0.05*	0.38±0.03*
3	25% v/v of ethanol	0.18±0.04	0.27±0.07	0.42±0.05
4	12% v/v of ethanol	0.17±0.03	0.36±0.04	0.53±0.02
5	5% v/v of ethanol	0.18±0.03	0.37±0.02	0.55±0.02

N=5, values were expressed as mean±SEM, (*) represent significant difference at $p<0.05$

Table 2 shows the result of the oxidative stress studies. The mean concentration of Malondialdehyde was significantly higher in group 2 when compared to the control ($p<0.05$). A significant decrease in the mean concentration of catalase was observed in groups 2 and 3 when compared to the control group ($p<0.05$). On the other hand, a significant decrease in the concentration of superoxide dismutase was observed in group 2 when compared to the control group ($p<0.05$). Although a dose dependent changes in the mean concentration of Malondialdehyde, Catalase and Superoxide dismutase was observed across the group following ethanol gavage.

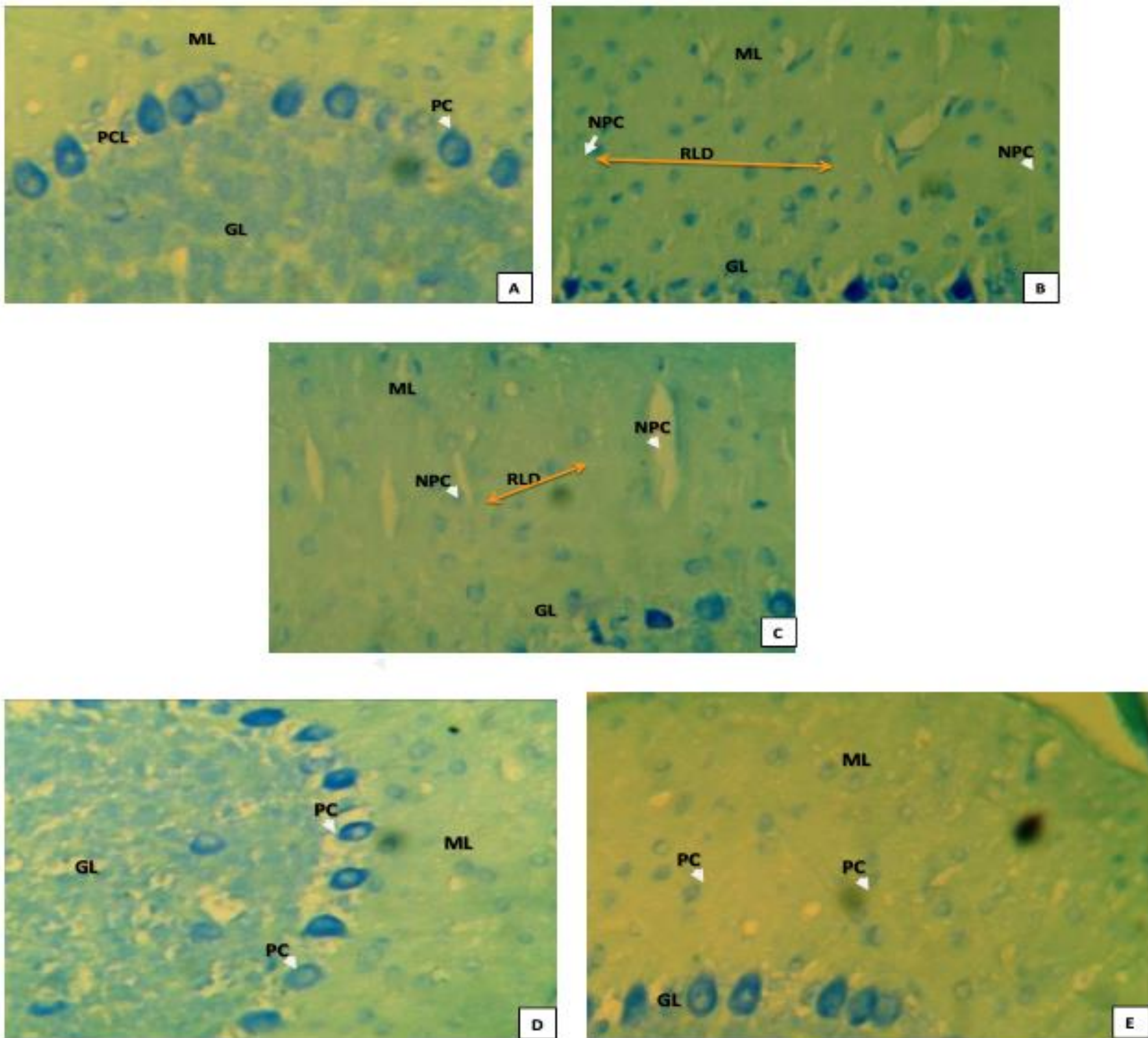

Figure 1: Photomicrograph of the control section (A), 40% v/v treated group (B), 25% v/v treated group (C), 12% v/v treated group (D) and 5% v/v treated group (E), all showing the three layers: Molecular layer (ML), Purkinje Layer (PL) and Granular Layer (GL). Necrotic purkinje cell (NP) and reduction of linear distribution of purkinje cells (RLD) was observed on plate B and C (Toluidin blue).

The result of the histological studies showed histological changes; reduced linear distribution of Purkinje cells and necrotic Purkinje cells, in section of the cerebellar tissue of rats in Groups 2 and 3 with sections from Groups 4 and 5 remaining relatively normal when compared to the slide from the control group.

Table 2: Effect of ethanol gavage on oxidative stress parameter in cerebellar tissues of adult wistar rats

Groups	Treatment	MDA (nmol/mg protein)	CAT (U/mg protein)	SOD (U/ml)
1	Control (distilled water)	28.93±2.49	0.73±0.07	46.27±3.06
2	40% v/v of ethanol	50.97±5.21*	0.33±0.07*	22.47±2.52*
3	25% v/v of ethanol	41.73±6.39	0.43±0.29*	31.83±1.39
4	12% v/v of ethanol	37.43±2.52	0.67±0.07	49.47±5.43
5	5% v/v of ethanol	33.23±4.79	0.83±0.03	53.03±6.38

N=5**,** Values are expressed as mean±SEM, SOD: Superoxide dismutase enzyme activity, CAT: Catalase activity and MDA: Malondialdehyde concentration. (*) represents significance p< 0.05

DISCUSSION

The cerebellum is the largest part of the hind brain, bulging back behind the pons and the medulla oblongata (Martin, *et al.*, 2002), involved in coordination of voluntary movement (Luo 2015). The cerebellum has been reported to be prone to the effect of neurotoxicants including ethanol (Riikonen et al, 2002). From the oxidative stress studies, the mean concentration of Malondialdehyde was significantly higher in group 2 when compared to the control (p<0.05). A significant decrease in the mean concentration of catalase was observed in groups 2 and 3 when compared to the control group (p<0.05). On the other hand, a significant decrease in the concentration of superoxide dismutase was observed in group 2 when compared to the control group (p<0.05). The variation in the oxidative stress parameter could be linked with the effect of ethanol on the cerebellar tissue. Free radical formation with an associated depletion of antioxidant enzyme (Calabrese *et al.*, 2002) and lipid peroxidation (Sun *et al.*, 2001; Ramachandran *et al.*, 2003) are the major pathways through which ethanol unleashes its effect on the central nervous system, thereby leading to oxidative stress (Warner and Gustafsson, 1994).Chronic ethanol consumption produces an increase in lipid peroxidation products such as malondialdehyde and a decrease in antioxidant factors, such as glutathione and its related enzymes (Albano, 2006).

The results of the sialic acid studies showed a dose dependent decrease in the mean concentration of bounded and total sialic acid concentration in the cerebellar tissue across the treatment groups, with group 2 having the lowest concentration followed by group 3. The observed depletion could be linked with cellular damage associated ethanol. Possible way of influence of ethanol on sialic concentration includes; depressed sailic acid synthesis or increased sialidase enzyme activities (Klemm and Engen 1979). Ethanol has the ability to penetrate lipid bilayer, therefore can expand and disorganize the interior of the membrane with resultant interfered membrane function (Goidstein, 1979; Klemm et al 1988). Total sialic acid content can be a potent indicator of oxidative stress in the brain tissues, even in ethanol induced generation of free radical (Tanaka *et al.* 1998; Usman et al, 2016).A single dose of ethanol (2 g per kg of body weight) given intraperitoneally to naive rats was associated with decreased sialic acid concentration within 45min in several brain region (Klemm and Engen 1979). Sialic acid is very important when it comes to proper membrane functionality and integrity (Angataand Varki, 2002). Previous studies have reported highest concentration of sialic acid in the brain where it participates as an integral part of gangliocide structure in neural transmission (Wang, 2006). Sialic acid is an immune moderator (Traving and Schauer, 1998) and appears to increase both memory and

cognitive performance (Morgan and Winick, 1980).

The result of the histological studies showed histological changes; reduced linear distribution of Purkinje cells and necrotic Purkinje cells, in sections of the cerebellar tissue of rats in Groups 2 and 3 with sections from Groups 4 and 5 remaining relatively normal when compared to the slide from the control group. The observed damage by ethanol is in line with reports of Courville (1966), who reported loss of Purkinje cells as a result of ethanol exposure. It was found that the continuous ethyl alcohol ingestion at high doses has risk effects on the cerebellum and its neurons especially Purkinje cells (Heaton et al., 2012; Hill *et al.,* 2011). Ethanol's harmful effects include neuronal cell death, impaired differentiation, reduction of neuronal numbers, and weakening of neuronal plasticity (Kumar, 2013). Ethanol-induced cerebellar damages persist even after complete abstinence from drinking (Luo, 2015). Permanent cerebellar deficits are often observed in alcoholics and the deficits persist even with abstinence from alcohol (Sullivan et al, 2002; Sullivan and Pfefferbaum 2005). Excessive alcohol exposure results in cerebellar ataxia and alterations in hand movements (Luo, 2015). Also, in the cerebellum, binge ethanol exposure has been shown to induce more severe damage in neonatal rats, than continuous consumption (Bonthius and West, 1990), and a loss of Purkinje cells has been reported after a single ethanol withdrawal in adult rats (Phillips and Cragg, 1984)

In conclusion, exposure to ethanol from the present studies showed a dose dependent effect on the cerebellum, as manifested in the histological and biochemical studies. We therefore recommend that studies on ethanol consumption should be given attention, since its effect cannot be neglected due to social and medical problem with its excessive consumption.

CONFLICT Of INTEREST: None

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