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Determination of Selected Trace Elements in the Cerebrum of Rat Pups (*Rattus norvegicus*) Following Prenatal Ethanol Gavage

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ABSTRACT

Of all the potential substances of abuse, ethanol (alcohol) is one of the most readily available. Most adults regularly or occasionally consume it. The most tragic effect of ethanol is on the central nervous system particularly during pregnancy. To assess this effect 6 pregnantly-timed dams divided into 3 groups were ingested with experimental equivalent concentrations of ethanol: low (8% v/v), medium (15% v/v) and high (45% v/v) via oro-gastric intubation procedure. A separate group was fed distilled water via same route. Administration of ethanol was done in the 2nd and 3rd trimesters of the rat gestational period. After natural delivery, cerebral tissues were obtained by dissection after a light anaesthesia followed by decapitation. The samples were analyzed for trace elements via an Instrumental Neutron Activation Analysis method (INAA) with the aid of a Nigerian Nuclear Research Reactor (NNRR-1). One way ANOVA was used to analyze the results at $p<0.05$. Game Howell and Tukey HSD post-hoc tests were used to check for the difference. Ca, Cu, Fe, Rb, Se, Zn, Na, Al and K were detected and quantified accordingly. Mean relative tissue concentrations for Cu, Rb, Zn, K and Al trace elements showed changes that were statistically significant at $p<0.05$ when compared to the normal control. No significant difference was found for Na, Fe, Se and Ca. Results from this study showed the teratogenic potential of ethanol on the developing cerebrum.

Keywords: *Trace elements, Instrumental neutron activation analysis, Cerebrum, Ethanol*

INTRODUCTION

Consumption of ethanol otherwise popularly called alcohol particularly during pregnancy can cause abnormal development of off-springs. Ethanol is a potential teratogen in pregnant females and its teratogenicity has been a subject of medical concern even from ancient times (Adebisi 2003).

So far the most tragic effect of prenatal ethanol ingestion is its effect on the developing brain tissue where it has been documented to cause a constellation of symptoms known as foetal alcohol syndrome (FAS), that is characterized by physical anomalies, growth retardation and central nervous system (CNS) dysfunctions (Clamp and Lindsley 1998; Bermann and Hannigan 2000). Even alterations in some neurochemical measurements can occur as a result of prenatal ethanol ingestion (Druse et al. 1990; Maier et al. 1996; Lebel et al. 2011). These neurochemical alterations can have significant consequences on the functions of the CNS because they modify the actions of neurotransmitters

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and can as well serve as neurotrophic and regulatory signals for normal brain development (Trombley and Shepherd 1996; Acuna-Castillo et al. 2000; Donaldson et al. 2003).

In this study, selected trace elements that play critical roles in the development of the CNS were investigated in the cerebrum of rat pups after prenatal ethanol ingestion by the dams by an Instrumental Neutron Activation Analysis method (INAA) with the aid of the Nigerian Nuclear Research Reactor (NNRR-1) at the Center for Energy Research and Training (CERT), Ahmadu Bello University, Zaria.

MATERIALS AND METHODS

Irradiation, Counting, Spectrum Evaluation and Quantitative Analysis

The animal use and care committee of the Directorate of Academic Planning and Monitoring Unit of Ahmadu Bello University approved the use of the animals in the research (research approval number ABUCAUC/2014/009), and animals were handled as stipulated by the guidelines for the use of animals for scientific research purposes.

Six (6) pregnantly-timed dams divided into 3 groups were ingested with experimental equivalent concentrations of ethanol low (8% v/v per kg bwt), medium (15% v/v per kg bwt) and high (45% v/v per kg bwt) via oro-gastric intubation procedure. A separate group was fed distilled water via same procedure and was labelled normal control and was assigned the number 1. The other experimental equivalent concentrations were assigned the numbers 2, 3 and 4 to represent low, medium and high doses respectively. Administration of ethanol was done in the 2nd and 3rd trimesters of the rat gestational period. After natural delivery, cerebrum were obtained from the rat pups by dissection following a light anaesthesia with ketamine hydrochloride injection at a dosage of 0.5 ml/kg body weight and then decapitation (5 pups were randomly selected from the 8% v/v and 15% v/v groups each, while only 4 pups were obtained from the 45% v/v group). The cerebrum were then dried at 50^{o} C in an oven (Memmert DINI ovum-280 KA) for 2 hours (IAEA 1980) and weighed with the aid of an electronic digital balance (Metler AE-240), and then transported to CERT for detection and quantification of trace elements.

The oven dried cerebrum (50 mg) were first wrapped in transparent polyethylene films, encapsulated into irradiation capsules and heat-sealed for neutron activation (irradiation).

INAA began with a neutron bombardment of the experimental samples including the normal control to convert stable isotopes to radioactive isotopes (IAEA 1980). The cerebral tissues were labelled with the letter 'B' (Biological sample). The NNRR-1 had 5 irradiation channels (B1-B5). Encapsulated samples B were hence labelled with numbers. Samples labelled with even numbers (B2, B4) were tested for long-live trace elements in the B2 and B4 irradiation channels of the reactor, while samples labelled with odd numbers (B1, B3 and B5) were tested for short-live trace elements in the B1, B3 and B5 irradiation channels of the reactor. The irradiations were done under an average neutron flux of 2.5×10^{12} n · cm^{-12} · s^{-1} and an operation power of 31 KW. The samples were irradiated for 5 minutes and counted for short-live trace elements. The count was repeated after a period of 600 seconds and the average of the two counts was taken. Irradiation for the long-live trace elements was done for 3 hours after 3 days and counts were taken. The count was repeated after 1 hour and the average count was also taken (IAEA,1980). Counts were performed based on gamma (γ) spectrometry measurement of induced radio nuclide (s) via a PC-based γ-ray spectrometry set-up. It consists of an N-type High purity Germanium detector (HpGe-coaxial type) coupled to a computer based multi-channel analyzer (MCA) via electronic modules. The energy resolution of the detector was 1.8 keV at a γ-ray energy of 1332 keV of ^{60}Co source. Gamma spectrum evaluation and quantitative analysis of each sample and control was done by a multipurpose γ-ray spectrum analysis software; WinSPAN version 2.10.

Data obtained was expressed as Mean ± SEM. One way ANOVA was used to analyze the results at $p<0.05$. Game Howell and Tukey HSD post-hoc tests were used to check for the difference.

RESULTS

The results of some trace elements in the cerebrum of rat pups after exposure to ethanol in-utero are presented in Figures 1-3. In Figure 1, the levels of zinc, sodium and potassium was analyzed in the cerebrum via Instrumental Neutron Activation Analysis technique. The result showed how ethanol interacted with the elements and caused decrease in the levels of these elements. These decreased changes were statistically significant ($p < 0.05$) for zinc and potassium. Likewise, in Figures 2 and 3, the levels of calcium, copper, iron, aluminium, rubidium and selenium were analyzed in the cerebrum via the same technique. The results showed decreases in the levels of these elements but the decreases which were only statistically significant ($p<0.05$) for copper, aluminium and rubidium.

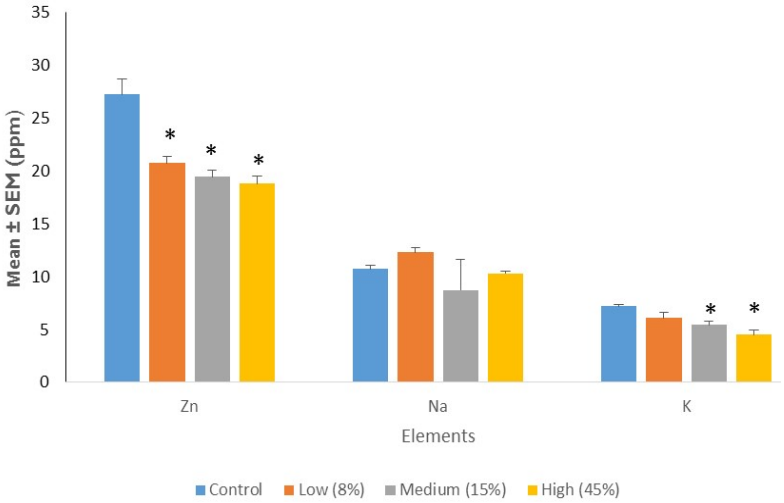

Fig. 1: Mean concentration of Zinc (Zn), Sodium (Na) and Potassium (K) in the cerebrum of rat pups. * indicate statistical significant difference ($p<0.05$) when compared to the normal control. ppm (part per million).

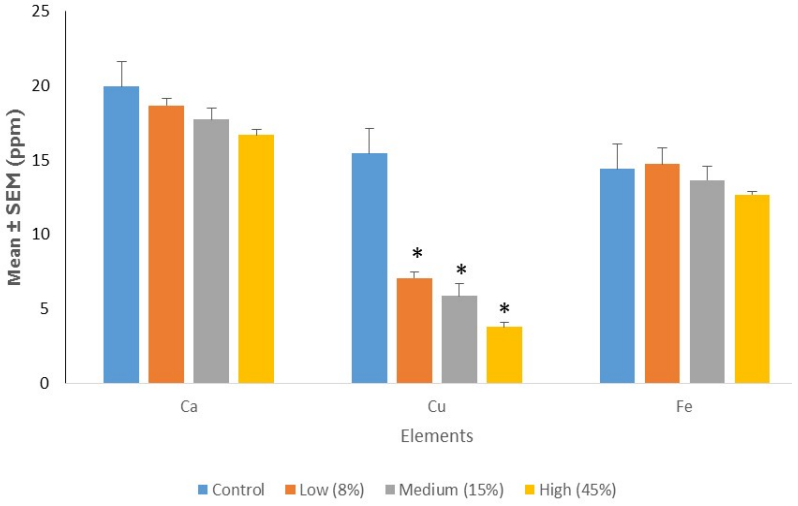

Fig. 2: Mean concentration of Calcium (Ca), Copper (Cu) and Iron (Fe) in the cerebrum of rat pups. * indicate statistical significant difference ($p<0.05$) when compared to the normal control. ppm (part per million).

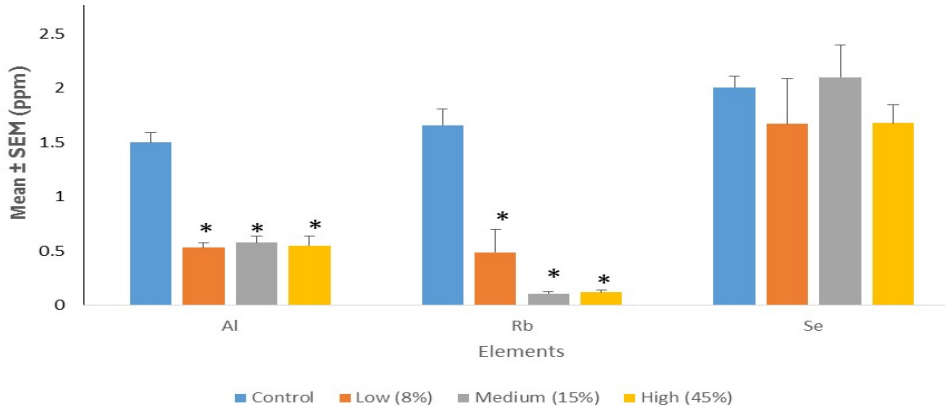

Fig. 3: Mean concentration of Aluminium (Al), Rubidium (Rb) and Selenium (Se) in the cerebrum of rat pups. * indicate statistical significant difference ($p<0.05$) when compared to the normal control. ppm (part per million).

DISCUSSION

INAA is a quantitative multi-element analysis which stands at the forefront of techniques for the quantitative analysis of major, minor, trace and even rare elements.

In all, nine trace elements were detected and quantified. These elements though in minute quantities are needed by the body to regulate the function, development and physiology of the CNS and by implication the well-being of the organism. Trace elements are involved in the regulation of neuronal gene expression and neuronal secretion and or modulation of neurotransmitter biosynthesis as well as serve as cofactors for numerous enzyme activities that are crucial for proper central nervous system development (Yakugaku 2004; Jacobus et al. 2011; Alexander et al. 2015). A deficiency of these trace elements can have far-reaching and long-lasting impact on development and activities of the cerebrum (Yakugaku 2004). Evidences from animal experimental data showed that prenatal ethanol exposure neurotransmitter pathways that depend on these trace elements were affected. For instance, evidences from the study by Tiwari et al. (2014) showed that glutamate and aspartate levels were reduced in the cerebral cortex of mouse brains exposed to ethanol *in utero* as well a differential decrease in the excitatory and inhibitory activities of neurotransmitters associated with GABAergic and glutaminergic neurons in the different cortical and sub-cortical brain regions of the mice. In an experiment on alcoholized rats in gestation and feeding. Shabanov et al. (2012) showed that alcohol affected the serotonergic and dopaminergic systems of the foetal rat brain resulting in the under-activity of these systems. In other animal studies, prenatal exposure to alcohol caused a reduction in hippocampal mass resulting in memory impairment (Bergmann and Hannigan 2000). Hypermethylation in the prefrontal cortex and hippocampus has been linked to prenatal ethanol

exposure in experimental rats (Otero et al. 2012). In a microRNA expression study on human as well as animal brains, Jacobus et al. (2011) reported impairment of this expression due to alcohol exposure thereby attributing to neuroinflammation and dysregulation of neurotransmission in the prefrontal cortex.

In this study, the teratogenic potential of prenatal ethanol ingestion was shown by the way ethanol influenced the concentration of trace elements in the cerebrum of the rat pups. Ethanol interacted and caused a decrease in the tissue concentrations of Cu, Zn, Rb, K and Al and these changes were statistically significant ($p < 0.05$). These trace elements are found in significant levels in the cerebrum and synaptic vesicles where they perform crucial roles in cellular metabolism and regulation of neurotransmission and neuronal gene expressions (Yakugaku 2004; Alexander et al. 2015). We found out that the prenatal ethanol exposure produced alterations in the concentrations of these elements and this can hamper normal brain development and function. This agrees with earlier reports by Druse et al. (1990), Maier et al. (1996) and Lebel et al. (2011).

In conclusion, the study has shown that prenatal ethanol ingestion interacted and induced a decrease in the concentrations of Cu, Zn, Rb, K, and Al in the cerebrum which may give rise to mental and neurological malfunctions.

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Conflict of Interest

None declared.

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