

Pathological Lesions in the Lungs of Neonatal Wistar Rats from Dams Administered Ethanol during Gestation

Sunday A. Musa, Shehu Ibrahim, Uduak. E. Umana, Samuel S. Adebisi and Wilson O. Hamman
Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria-Nigeria

Abstract: This study was carried out to investigate the effects of ethanol ingestion during pregnancy on the fetal lungs development. Adult Wistar rats were used and grouped into four groups and each group having four females and two males. Group A was the control group received only distilled water, while groups B, C and D received 0.2 mL of 20, 25 and 30% ethanol orally respectively daily for seven days during the 4th to 10th day of gestation. After delivery, the fetal lungs were removed and fixed in 10% buffered formalin. The neonates' lungs were prepared through histological techniques and stained with Haematoxylin and Eosin and were studied under the light microscope. The result showed alveolar degeneration, bronchiole-capillary thickening, bronchiolar degeneration and extravasations of erythrocyte in the ethanol treated groups while the control was normal. Ethanol ingestion during pregnancy could lead to ethanol-induced lung damage in the fetuses. Hence, alcohol ingestion should be avoided during pregnancy.

Key words: Ethanol, neonatal lungs, wistar rats

INTRODUCTION

Alcohol abuse causes injuries of multiple organs and tissues affecting the brain, lungs, liver, cardiovascular system, immune system (Fillmore, 2003; Frank *et al.*, 2004). Prenatal ethanol exposure can potentially jeopardize both maternity and embryo. Fetal Alcohol Syndrome (FAS) is a serious injury to fetus and child caused by maternal prenatal exposure (Jones and Smith, 1973). Ethanol is a known teratogen in humans and its ingestion by pregnant women is the primary cause of Fetal Alcohol Syndrome, (FAS) as well as the milder manifestation known as Fetal Alcohol Effect (FAE) (Maitson and Reley, 1998). The decrease in mental capacity and delayed maturation following fetal alcoholic exposure are associated with abnormality in number and structures of neurons throughout the cerebral cortex and other parts of the brain (West and Pierce, 1996; Ahveninen *et al.*, 2000; Sampson *et al.*, 2000). Ingestion of fermented beverages containing ethanol has been known since the beginning of recorded history (Keith and Andrea, 1996). It was first thought to have strong medicinal properties, but later recognized that its therapeutic value is extremely limited and it is now known that chronic consumption of excessive amount of alcohol is a major source of social and medicinal problems (Keith and Andrea, 1996).

In humans, ethanol has a neuropathic effect primarily when administered after the first twenty weeks in utero.

The major effects of ethanol are thought to be mediated by ethanol itself, since in animal models, most of ethanol primary metabolite, acetaldehyde is metabolized by the placenta (Michaelis and Michaelis, 1986). However, recent studies showed that rats' fetal brain tissue can itself form acetaldehyde from ethanol via catalase. Suggesting that acetaldehyde may play role in the fetal brain response to ethanol. Prenatal exposure also causes significant decrease in birth weight, microcephaly and memory deficits, learning and behavioural abnormality in rat pups raised under FAS model system, as well as altered neurotransmitter level (Barnes and Walker, 1981).

Although alcoholism and its associated complication is one of the most common medical disorders (Nathan, 2006, unpublished). Current understanding of the neurobiological mechanism responsible for the diverse clinical manifestations of human alcoholism remains rather primitive. Ethanol is capable of generating oxygen radicals inhibiting glutathione (GSH) synthesis, inducing reduced glutathione levels in the tissue, increasing malondialdehyde (MDA) levels and impairing antioxidant defensive mechanisms in humans and experimental animals (Guidot and Duncan, 2002). The lungs are one of the target organs most vulnerable to oxidative stress due to their unique structure and function (Lang *et al.*, 2002). Because prenatal ethanol exposure has become a widespread social problem, scientists are now studying the alcohol-related lung disorders with great interest. This study was carried out to study the effect of ethanol ingestion during pregnancy on the neonatal lung development in Wistar rats.

MATERIALS AND METHODS

Animals: This study was conducted in February 2009 in Human Anatomy Department, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. Twenty-four (24) adult male and female Wistar rats bred in the Faculty of Veterinary Medicine Ahmadu Bello University, Zaria, Nigeria, of average weight of 188g were used for this study. They were kept in the animal house of the Department of Human Anatomy, Faculty of Medicine and were allowed to acclimatize for three weeks. They were allowed to have free access to food and water. After three weeks of acclimatization, they were grouped into four groups and each having four (4) females and two (2) males. This was done based on the knowledge of the estrous cycle which is between 5-8 days. After mating overnight vaginal smear was taken and viewed under the light microscope to confirm copulation. The microscopic examination revealed that all the animals mated.

Group A served as the control group which received distilled water orally while groups B, C and D were administered 0.2 mL of 20, 25, 30% ethanol orally respectively, starting from the 4th day of day to the 10th day of gestation targeting pseudo-glandular phase and early canalicular phase compared to human embryology which occurs at (15th-16th week) and (16th-26th week) of intrauterine life respectively (Sadler, 2006).

On the 21st day of gestation, 20 and 30% ethanol treated groups littered while the 25% ethanol treated group littered on the 23rd day. The fetuses were observed physically and sacrificed immediately through light chloroform inhalation and fixed in 10% buffered formalin and further processed histologically using the bench model of automatic tissue processor, available in the histological section of the Department of Human Anatomy, Faculty of Medicine, A.B.U., Zaria, Nigeria. The slides were examined under the light microscope at the magnification of x25 and photomicrographs were taken using AmScope digital camera (model number MD900).

RESULTS

The control group showed normal bronchiolar and alveolar structures as shown in Fig. 1, while ethanol-treated groups showed some degree of distortions. Group B (20%), the neonatal lungs from this group showed congestion, bronchiolar and alveolar degeneration, alveolar-capillary thickening and extravasations of erythrocytes as seen in Fig. 2. Group C (25% ethanol) also showed bronchiolar and alveolar structural degenerations, there was alveolar capillary thickening and extravasations of erythrocytes as seen in Fig. 3. Group 4 (30% ethanol) showed more degeneration of the bronchioles and alveolar structures, alveolar capillary thickening and extravasations of erythrocyte as seen in

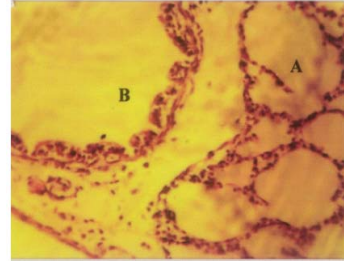


Fig. 1: Photomicrograph of control (Group A) showing normal bronchiolar (B) Alveolar (A) structure of the lung, H & E x 25

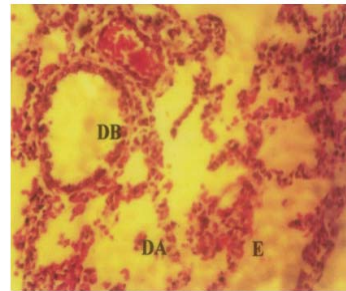


Fig. 2: Photomicrograph of 20% ethanol (Group B) showing Degenerated Alveolar (DA) structures, Degenerated bronchiole (DB) and Extravasations (E) of erythrocytes H & E x 25

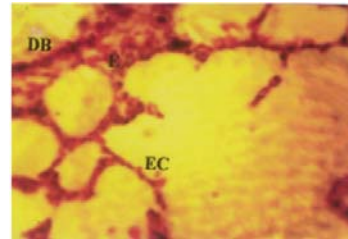


Fig. 3: Photomicrograph of 25% ethanol (Group C) showing Emphysematous Changes (EC) of alveolar structures, Degenerated bronchiole (DB) and Extravasations (E) of erythrocytes, H & E x 25

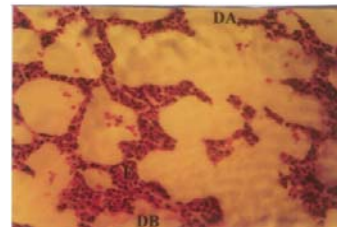


Fig. 4: Photomicrograph of 30% ethanol (Group B) showing Degenerated Alveolar (DA) structures, Degenerated bronchiole (DB) and Extravasations (E) of erythrocytes H & E x 25

Fig. 4. All these were seen in the lungs of the fetuses of the ethanol treated groups B, C and D.

DISCUSSION

The control group has shown normal bronchiolar and alveolar structures. In the ethanol treated groups B, C and D, the effect observed were degenerations of the bronchiolar and alveolar structures, thickening of alveolar capillary walls and extravasations of erythrocytes. Thickening of alveolar capillary wall that forms the blood-air barrier causes extravasations of erythrocytes into the lung tissues and this could lead to hemorrhage in the lungs. The great alveolar cells (type II pneumocytes) which synthesize and secrete phospholipids rich product called pulmonary surfactant are deficient in areas of degenerated alveolar structures. The function of surfactant to spread over the alveolar cell surface to moisten them and lower the alveolar surface tension is diminished in the ethanol treated groups. In this manner, the function of surfactant to stabilize alveolar diameters and prevent their collapse during respiration by minimizing the collapsing force is diminished which could lead to collapse of the lungs. This present findings agrees with the work done by (Aytacoglu *et al.*, 2006), on alcohol-induced lung damage and increased oxidative stress in which histopathological examination demonstrated thickening of alveolar capillary, degeneration of alveoli, leukocyte infiltration. However, in this present work, the degeneration was much more severe but no leukocyte infiltration was observed. Furthermore, some of the neonates that died in the ethanol groups, this could likely be due to surfactant deficiency that are secreted during the last week of gestation. Surfactant is important at birth, the fetus makes respiratory movements in utero, but the lungs remain collapsed until birth. The degeneration in the ethanol treated groups could likely be the possible cause of surfactant deficiency that leads to collapse of the lungs (Ochs, 2006).

From the results observed in this study, it is concluded that ethanol ingestion during pregnancy has degenerative effects on the fetal lungs and therefore could lead to lung collapse (Ochs, 2006). Ethanol can have a detrimental effect on this lung protective mechanism depending on the length of exposure. When acutely-exposed to ethanol, the ciliary beat frequency in bovine bronchial epithelial cells was increased (Wyatt *et al.*, 2003). Interestingly, when chronically exposed to ethanol, the effect was quite opposite and ciliary beating was decreased (Wyatt and Sisson, 2001). This chronic desensitization of cilia stimulation by ethanol has also been demonstrated in vivo in rat (Wyatt *et al.*, 2004) and mouse (Elliott *et al.*, 2006) models.

Surfactant deficiency may lead to infant respiratory distress syndrome (IFDS), the serious pulmonary disease that develops in infants born before their surfactant

system is functional that lead to high surface tension in the lungs and alveoli are collapsed in many areas as observed in the ethanol treated groups. Alcohol consumption during pregnancy has been associated with many harmful effects in the developing fetus. Most studies have focused on neurodevelopment (Riley and McGee, 2005; West and Blake, 2005) and behavioral anomalies (Mattson *et al.*, 2001; Sood *et al.*, 2001), in infants born from mothers who drank during pregnancy.

CONCLUSION AND RECOMMENDATIONS

It is therefore recommended that pregnant women should avoid ingestion of alcohol. Hence, precautions against infant respiratory distress syndrome may prevent morbidity and mortality in alcohol-induced lung damage.

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