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MORPHOLOGY OF THE LIVER OF WISTAR RATS EXPOSED TO ALCOHOL DURING PREGNANCY AND LACTATION

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ABSTRACT

Aim: Maternal alcohol intake during pregnancy and/or lactation results to some developmental defects in the offspring including growth retardation. Information is limited on the effect of alcohol on the liver of dams exposed to alcohol.

Methods: Rat model was employed to investigate the effects of alcohol on the liver of the dams. 75 female Wistar rats grouped into 3 of 25 each were used. The rats were later bred overnight after acclimatization for three weeks by introducing one male rat into a cage housing five females. Pregnancy was presumed after observing vaginal plug the following morning. Group 1 served as control (C), Groups 2 and 3 were exposed to alcohol during pregnancy and lactation (APL) and during lactation only (AL) respectively. At Days 7, 14, 21, 35 and 49 postpartum, 5 lactating rats were selected from the three groups and sacrificed and the liver dissected out, weighed, fixed in 10% formalin and prepared for routine histological examination.

Results: The results showed significant increase ($p<0.05$) in the weights and degeneration of hepatocytes of the liver.

Conclusion: Our findings suggest that alcohol intake during pregnancy and/or lactation is injurious to the liver of dams which could lead to impairment of foetal growth.

Key Words: Alcohol, Pregnancy, Lactation, Liver, Hepatocytes

INTRODUCTION

The consumption of alcohol is firmly established in the society probably since the accidental discovery of fermentation by yeast (Cornwell and Cornwell, 1997). Statistics indicate that alcohol consumption is on the increase (Van-Beer, 2004) and is consumed by people for a variety of reasons. Such reasons include entertainment and as a means of reducing stress and emotional upset which are commonly observed in pregnant women (Nucleus Medical Art, 2004). In adult humans and experimental animals, alcohol is known to be injurious to various organs and tissues (Rothmans, 1980, Grail et al., 1992, Ballard, 1997, Zoeller, 2002). It has been observed that alcohol consumption during pregnancy leads to foetal alcohol syndrome (FAS) in humans and experimental animals. Characteristics of this syndrome include growth deficiency, microcephaly and central nervous system dysfunction (Scraag, 1995). Since the recognition of FAS, numerous animal studies over the years have demonstrated the deleterious effects of alcohol consumption during pregnancy and/or lactation on the offspring (Ihemelandu, 1984, Onu et al., 2003, 2004, Onu et al., 2011), with little attention paid to the

organs of the dams which could be affected by alcohol. There is paucity of data in the literature on the effects of alcohol on the liver of pregnant experimental animals exposed to alcohol during pregnancy and/or lactation. The aim of this study is to evaluate, using rat model, the effects of alcohol on the morphology of liver in rats exposed to alcohol during pregnancy and/or lactation.

MATERIALS AND METHODS

Experimental Animals

Seventy five female Wistar rats aged 9 - 10 weeks were used in this investigation. The rats were obtained from the Laboratory Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. All the animals were fed with water and commercial diet (Guinea Feed, Bendel Feed and Flour Mills, Nigeria Plc.) ad libitum throughout the duration of the study. After acclimatization for five weeks, the 75 female rats were divided into 3 groups of 25 each. Group 1 served as control (C), Group 2 was exposed to alcohol during pregnancy and lactation (APL) while Group 3 was exposed to alcohol during lactation only (AL).

Breeding of Experimental Rats

At the commencement of the study, the female rats were bred overnight by introducing 1 male rat into a cage housing 5 female rats. Day 1 of pregnancy was presumed after observing copulation plug the following morning (Nwaogu and Ihemelandu, 1999).

Alcohol Administration

Following pregnancy detection by observation of the vaginal/copulation plug (Nwaogu and Ihemelandu, 1999), 2g/kg body weight of 30% ethanol (v/v) was given to the pregnant rats in APL per os according to the method of Maier and West, (2004). Theses continued throughout pregnancy and lactation. After delivery, the lactating rats in AL were given the same quantity of ethanol per os and this lasted throughout lactation period.

Sample Collection, Quantitative Measurements and Histopathology

At Day (D) 7, 14, 21, 35 and 49 postpartum, 5 lactating rats each from the 3 groups (Control, APL and AL) were sacrificed and their liver removed and weighed using Metler's Analytical Balance (MICROWA SWISS 5540) and expressed as relative weights according to Riser and Shirer (1967). Thereafter, the liver tissues were dehydrated through a graded series of ethanol (50%, 70%, 90%, 95% and 100%) cleared in xylene, infiltrated with paraffin, embedded in fresh molten paraffin. Sections, 4µm thick were stained with haematoxylin and eosin (H&E). Photomicrographs were then produced.

Statistical Analysis

The data generated from the measurements were subjected to statistical analysis. Means and standard errors of means (means ± SEM) were calculated for each group. Statistical differences between the mean values of the relative weights of liver were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) (Duncan, 1955). For the statistical analysis, $P<0.05$ was considered statistically significant.

RESULTS

The relative weights of liver were significantly increased ($p<0.05$) in APL and AL at D7, D14, D21 and D35 relative to their control group counterparts (Table 1). The liver of the control showed normal liver histological structure throughout the period of the study with hepatic cords radiating from the central vein while the liver of APL showed vacuolar degeneration of hepatocytes at D21 postpartum (Fig.1).

DISCUSSION

Alcohol in this investigation provoked hepatomegally in Wistar rats exposed to it during pregnancy and during lactation. The reason for the enlargement of the liver was not known for certain. The liver is the primary site for alcohol metabolism, and it is particularly susceptible to alcohol-related injury (Boggan, 2003). Alcohol intake puts a great metabolic overload on the liver and the response to this overload could lead to increase in weight. The liver prefers alcohol as an energy source and stops the use of fat which accumulates (Cornwell and Cornwell, 1997). Again, the hydrogen produced as a by-product of alcohol metabolism is converted to more fat for the synthesis of cholesterol and lipoprotein which accumulates as fat droplets in the liver (Cornwell and Cornwell, 1997). All these could

Table 1. Liver of lactating Wistar rats exposed to alcohol during pregnancy and/or lactation

Lactational period (Days)	Control	APL	AL
7	3.40 ± 0.15^{a}	5.88 ± 0.10^{b}	4.72 ± 0.46^{c}
14	3.62 ± 0.10^{a}	6.84 ± 0.27^{b}	5.08 ± 0.10^{c}
21	3.54 ± 0.07^{a}	6.92 ± 0.23^{b}	4.90 ± 0.30^{c}
35	3.50 ± 0.10^{a}	5.98 ± 0.01^{b}	4.18 ± 0.001^{c}
49	3.70 ± 0.10	3.54 ± 0.21	3.69 ± 0.10

Mean with different superscript on the same row are significantly different ($p<0.05$)
APL: Rats exposed to alcohol from pregnancy to lactation
AL: Rats exposed to alcohol during lactation only

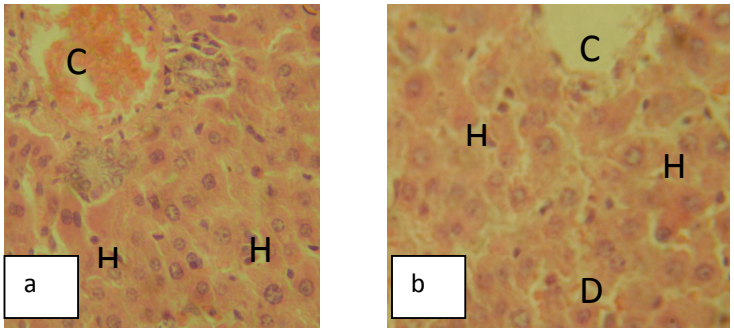

Plate 1: Photomicrograph sections of liver of lactating Wistar rats exposed to alcohol.
(a) Control showing normal liver histology with hepatic cords radiating from the central vein. This was also observed in AL.
(b) Liver of dams (APL) at D 21 postpartum showing vacuolar degeneration of hepatocytes (DH).
HC = Hepatic cords, CV = Central vein, HS = Hepatic Sinusoids. X600, H&E

be responsible for the hepatomegally observed. However, at D49, the weight of the liver in APL and AL did not differ from that of the control. This could be due to recovery by the organ from the effect of alcohol following withdrawal, because once alcohol intake ceases, the accumulated fat soon disappears from the liver and the organ returns to normal (Cornwell and Cornwell, 1997). The degeneration of hepatocytes observed in the liver of rats exposed to alcohol during pregnancy and lactation could have been due to oxidative stress. Oxidative stress induces apoptosis and necrosis of cells. It has been documented that oxygen free radicals which are products of alcohol metabolism (Cornwell and Cornwell, 1997) react with unsaturated membrane lipids, initiating a self-perpetuating peroxidation process (Lipid Peroxidation, 1989). This reaction can produce loss of membrane function and ultimately cell death. Naturally there are scavenger molecules known as antioxidant (glutathione) that are normally found within the cells which eliminate free radicals (Goodlett and Horn, 2001). Unfortunately, alcohol depletes this antioxidant and when this happens, oxidative stress occurs. In this investigation, the alcohol administered could have yielded free radicals during its metabolism and may also have depleted the naturally occurring antioxidant leading to above effects. This has been observed in fetal liver (Devi et al., 1993). Alcohol also causes reduction in the activity of alcohol dehydrogenase and cytochrome P-450 in the liver that are involved in alcohol metabolism. The reduced activity of these enzymes leads to accumulation of alcohol and subsequent death of cells due to the toxic effect of alcohol. The results of this investigation suggest that alcohol consumed during pregnancy and/or lactation could be harmful to the liver of the dams which could ultimately impact on the growth of the offspring considering the role of the liver in the biochemical activity of the body.

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