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Comparative study of teratogenic effects of brewed beer and palm wine on fetal femur bone of albino Wistar rats

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ABSTRACT: Alcohol consumption has adverse effects on both adult and developing bones. The mechanism by which alcohol affect bone, however is unknown. The purpose of this study was to compare the effects of industrial brewed beer and palm wine on fetal femur bone. Twenty virgin female Wistar rats weighing 180-200g were used in the study. The rats were equally divided into five groups of A, B, C, D, and E. Group A served as the control, while B, C, D, and E were the experimental groups. Female rats at proestrous, determined by vaginal lavage were caged overnight with sexually matured males of the same strain. Following pregnancy, groups B and C were respectively given 4mls and 8mls of brewed beer, while groups D and E were respectively given 4mls and 8mls of fermented palm wine from 7^{th} -13^{th} day of gestation. Fetuses were collected on day 20 of gestation and the fetal bones extracted and preserved in buffered formalin, decalcified using 4% formic acid and processed for routine H and E stain. Histological study of the epiphyseal plate of the fetal bones showed reduced intercellular matrix, scattered and vacuolated cartilage cells in the experimental groups. These effects were dose-dependent and were more pronounced in the groups that received the brewed beer. These results suggest that prenatal alcohol exposure may have osteo-inhibitory effects on bones, and that industrialized brewed beer is more toxic to the developing bone.

Keywords: Palmwine, Industrialized brewed beer, Fetal bone, Albino rats.

Introduction

Industrialized brewed beer and palm wine are alcoholic beverages considered to play an indispensible role in local ceremonies such as traditional marriages and other social ceremonies in Nigeria. They are consumed by both men and women including pregnant women (Sixth special report-the U.S. Congress on alcohol and health, 1987). The percentage of alcohol in industrialized brewed beer is documented to be 6.1%, while that of fermented palm wine was estimated to be 4.4%.

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Alcohol is a low molecular substance and is therefore capable of crossing the placental barrier, causing the level of alcohol in the mother to be approximate to that of the fetus (Stressquth *et al*, 1998). Fetal alcohol syndrome is one of the leading causes of mental retardation and is directly attributed to drinking alcohol during pregnancy. It is characterized by growth retardation, facial abnormalities, central nervous system dysfunction as well as behavioral problems (CDC, NCBDD/FAS, 2004). Partial expression of these patterns is seen fetal alcohol effects (Epstein and Sycheston, 1987).

The effect of alcohol is determined by the rate at which it is absorbed into the body, which depend on the kind of alcoholic beverage consumed, the proportion of alcohol it contains, the speed with which it is drunk and the amount and type of food in the stomach as well as on certain minor physiological difference among individuals (Weinber *et al*, 1990). The magnitude of the risk is increased because alcoholic beverages are very popular in our society, and is well consumed by both educated and illiterate females.

The habitual consumption of even moderate quantities of alcoholic beverages is clearly associated with reduced bone mass, increased prevalence of skeletal fracture and a major risk factor for the development of secondary osteoporosis. The bone is a tissue which is active throughout life (Rosse & Gaddum- Rosse, 1997), and may be prone to adverse effects. This study therefore is designed to compare the possible teratogenic effects of the alcoholic content of star beer and palm wine on developing femur bone.

Materials and Methods

Twenty five virgin female Wistar rats weighing 180-200g were used for this study and the animals were bred in the animal house of the Department of Anatomy, University of Calabar. The rats were randomly divided into five groups – A, B, C, D and E, each consisting of five female Wistar rats. Female Wistar rats at proestrous, determined by vaginal lavage were caged overnight with sexually matured males of the same strain. The following morning, vaginal smear obtained were observed under the light microscope and presence of sperm indicated zero day of pregnancy.

Following pregnancy, rats in groups B and C were given 4ml and 8ml of industrial brewed beer respectively, while groups D and E were given 4ml and 8ml of palm wine respectively from day 7^{th} – 13^{th} of gestation. The animals were sacrificed on the 20^{th} day of gestation using chloroform inhalation method. The fetuses were removed by uterectomy. The fetal femurs were extracted with the aid of a sharp needle and a forceps. The femurs were then fixed in buffered formalin, and then decalcified using 4% formic acid. Complete decalcification was tested by using ammonium oxalate solution which when dropped in the decalcifying agent turns cloudy if decalcification is not complete. Routine histological processing was carried out and stained using H and E methods.

Results

Histological study of the epiphyseal plate (proliferating zone) of the fetal femur bone of the control group showed rich intercellular matrix (IM) and numerous cartilaginous cells (CC) arranged in columns (Plate 1).

Intercellular matrix between the cartilaginous cells were greatly reduced, the cartilage cells in the proliferating zone were scattered and some vacuolations were seen in groups B and C which received 4mls and 8mls of brewed beer respectively (Plate 2 and 3). The effects were more pronounced in group C which received 8mls of the brewed beer.

In groups D and E animals that were given 4mls and 8mls of palm wine respectively, the cartilaginous cells in the proliferating zone were scattered, few were vacuolated with reduced intercellular matrix. This was more pronounced in the group that received 8mls of palm wine. The adverse effects were more pronounced in the groups that received brewed beer compared to the groups that received palm wine.

PHOTOMICROGRAPHS

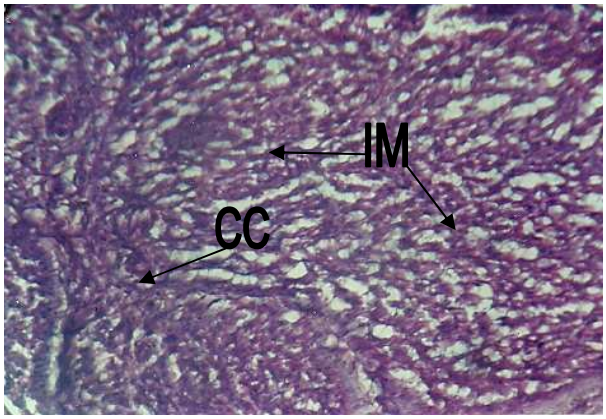

Plate 1: The micrograph showing the proliferating zone of the fetal femur bone of the control group with rich intercellular matrix (IM) and numerous cartilaginous cells (CC) arranged in columns. Mag x400. H & E

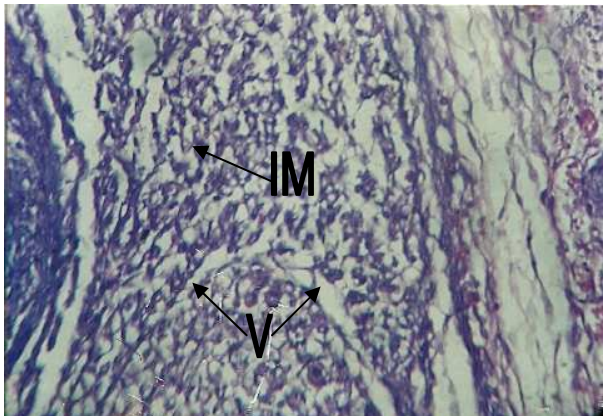

Plate 2: Group B micrograph treated with 4mls of brewed beer showing greatly reduced intercellular matrix (IM) between the cartilaginous cells, the cartilage cells in the proliferating zone are scattered, and some vacuolations (V) are seen. Mag x400. H & E

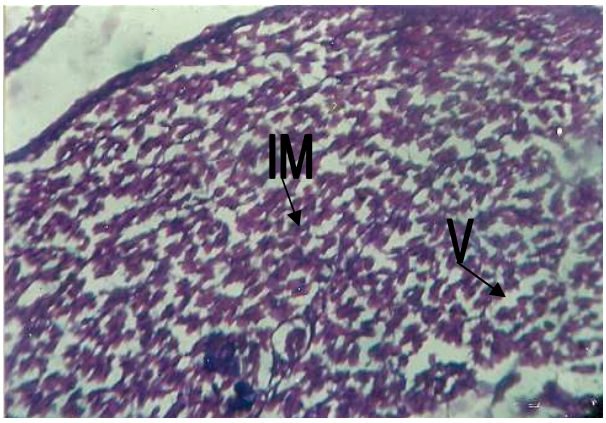

Plate 3: Group C micrograph treated with 8mls of brewed beer showing greatly reduced intercellular matrix (IM) between the cartilaginous cells, scattered cartilage cells in the proliferating zone, and some vacuolations (V) are seen. Mag x400. H & E

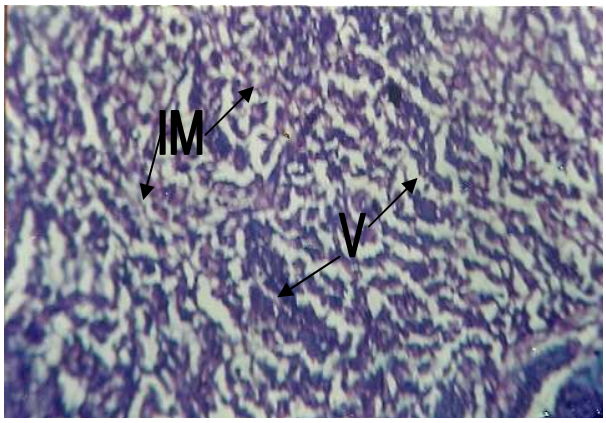

Plate 4: Group D micrograph treated with 4mls of palm wine showing scattered cartilaginous cells in the proliferating zone, less vacuolation (V), with reduced intercellular matrix (IM). Mag x400. H & E

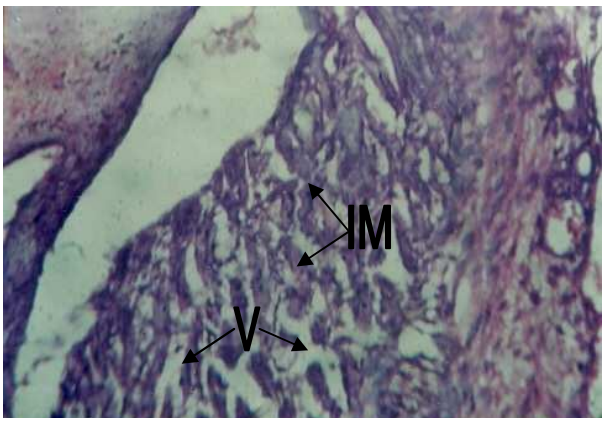

Plate 5: Group E micrograph treated with 8mls of palm wine showing pronounced scattered cartilaginous cells in the proliferating zone, less vacuolation (V), with reduced intercellular matrix (IM). Mag x400. H & E

Discussion

Alcohol is the most frequent and most important teratogenic noxa for the embryo and fetus (Schilgen and Loeser, 1994). Alcohol consumption have adverse effects on both adult and developing bone. The mechanisms by which alcohol affects bone however are unknown (Keiver *et al*., 1997).

Histologically, the cellular arrangement in the proliferating zone deviated from the normal arrangement of cells in columns. The intercellular matrix between the columns was greatly reduced, and this may be due to the osteo-inhibitory effect of chronic alcohol exposure possibly due to the inhibition of cell proliferation and mal-differentiation of mesenchymal cells in the tissue (Chakklakal, 2005). Many teratogens exert their effects on the proliferating cells by interfering with cell division (Barson, 1973). Lee and Leichter (1983) showed that alcohol caused skeletal retardation and the dimensions of the ossification centers in the limbs were less in the alcohol fetuses than in the controls.

Chronic and heavy drinking disrupts the normal balance between bone formation by osteoblasts and the removal of old bone by osteoclasts. Alcohol induced osteopenia has characteristics that are different from other disease states involving bone loss (Chakkalakal, 2005). IL – 1, a bone regulating cytokine has been shown to exert direct effect in skeletal cells (Roodman, 2001, Roux and Orcel, 2000). An effect on the cell has been reported by Oreffo *et al* (2003). It was reported that IL – 1 affect the offspring in an indirect manner via the placenta. IL – 1 exposure also resulted in decreased skeletal growth and a reduced amount of cortical bone in adult rat offspring (Swolin-Edie *et al*., 2004). Keiver *et al* (1997) reported that prenatal ethanol exposure has effect on the resting zone of the developing bone indicating that early stages of bone development may also be disrupted.

The histological findings of this study therefore suggest that prenatal alcohol exposure may have osteo-inhibitory effects on bones, and that industrialized brewed beer is more toxic to the developing bone.

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