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The Histomorphological Studies Of Viscera In Mice Foetal Alcohol Syndrome

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ABSTRACT

This work studied the effect of prenatal alcohol exposure on histomorphology of the heart, spleen and lungs of mice pups. Twenty-two 8-week old female inbred albino mice were divided into two groups of 11 each. Group 1 was given 10% ethanol (v/v) in water for two weeks, then increased to 20% (v/v) for another three weeks. Group 2, which served as control received ordinary water throughout the period of the experiment. The animals in both groups were randomly mated overnight. On diagnosis of pregnancy, the ethanol was increased to 30% (v/v) for group 1 and maintained on this until birth when it was replaced with water. Both groups were fed commercial diet *ad libitum*. At the 4th day of age, the pups were sacrificed and the heart, spleen and lungs carefully dissected out, histosections prepared and stained with H & E. The histomorphological changes observed in the alcohol exposed pups included oedema of interaveolar sepatae, oedema and fatty degeneration of myocardiac fibers. Their spleen was reactive and contained more lymphoblasts and lymphocytes while that of control contained more red blood cells.

Key Word: Alcoholism. Histomorphology. Mice pups. Viscera

Foetal Alcohol Syndrome (FAS) is defined as a characteristic set of symptoms appearing in mothers who drink alcohol during pregnancy (Maisto *et al* .,1999). The pattern of prenatal and postnatal effects of alcohol on the foetus were first described by Lemoine *et al.* (1998) and Jones *et al.* (1973). It has been demonstrated that ethanol ingested by the mother crossed the placenta and reached the foetus in concentrations similar to that found in the material circulation (Mann, 1975; Romert and Mathiessen, 1992). The excessive consumption of alcohol during pregnancy is common in many countries in spite of the fact that this represents a significant risk to normal intra and extrauterine development of the embryo and feotus at various stages of pregnancy. In a recent study in United States of America only 72% of respondents have heard of FAS and more than 33.3% incorrectly reported that it describes a baby born addicted to alcohol and that the syndrome can be inherited and cured (Mackinnon *et al.*, 1995). This awareness will be much less in developing countries including Nigeria.

In human and experimental animal models, FAS is characterized during neonatal life by central nervous system dysfunctions, growth deficiencies, a typical facial appearance and various system malformations, (Ramanathan et al., 1996). There was also muscle hypotropgy (Ihemelandu, 1984) as well as retardation of allometric growth of skeletal muscles and various viscera (Nwaogu and Ihemelandu, 1999a and b).

Reports on FAS have focused mainly on the histomorphology of the brain, liver and kidney (Assadi *et al.*, 1992; Devi *et al.*, 1993; Miller, 1993 and Riley *et al.*, 1995). There is dearth of information on the effect of prenatal alcohol exposure on histomorphology of other viscera either in human or experimental animals. This work therefore was designed to study the histomorphology of lungs, heart and spleen in mice pups exposed to alcohol prenatally.

MATERIALS AND METHODS

The mice used for the study were obtained from a colony of inbred albino mice maintained in the animal house of the Faculty of Veterinary Medicine, University

of Nigeria, Nsukka. They were housed in cages with screened tops and kept for two weeks acclimatization period.

22 eight weeks old female albino mice were randomly divided into two groups of 11 each. Group 2 served as control. The experimental group 1 was given 10% ethanol (v/v) in drinking water for two weeks, then 20% ethanol (v/v) for another three weeks. Animals in both groups were randomly mated. Day 1 of pregnancy was established by observing vaginal plugs and sperms in vaginal washing the next morning. The alcohol was adjusted to 30% (v/v) and pregnant animals in group 1 received this till delivery when it was replaced with ordinary water. Both groups were fed commercial diet *ad libitum* throughout the period of the experiment. Mortality of the pups was recorded and the rate calculated in both groups. At 4 days of age pups from both groups were sacrificed by severing the spinal cord at the atlanto-occipital junction. The lungs, heart and spleen were dissected out and fixed in Bouin's fluid. These organs were prepared for sectioning and embedded in paraffin wax. Sections of 4u thickness were cut using microtome and stained routinely with Haematoxylin and Eosin. The sections were studied and photomicrographs prepared.

RESULTS AND DISCUSSION

The result of this study suggest that consumption of alcohol during pregnancy produced adverse effects on the development of the foetus and neonates in mice. This was evident by high mortality rate observed in the alcohol-exposed pups (Table 1). This observation further supports the report that alcohol induces foetal death (Chernaff, 1977; Ranadall *et al.*, 1977). This corroborates the observations of Middaugh and Boggan (1995) who reported 28.9% and 30% mortality rates in alcohol exposed pups as against 0% in the control.

Blood capillaries supplying the alveoli are found in the interalveolar septae. Distension and cellular infiltration of these septae in the alcohol exposed pups (Fig. 1) is likened to interstitial pneumonia and may be due to damaging effect of alcohol or its metabolites on the capillaries. This agrees with the observation that alveolar septal damage is caused by blood-borne chemicals or insults in most instances (Dungworth, 1993). It may be oedema resulting from anemia, since anemia is one of the effects of alcoholism. Degeneration and necrosis of the myocardiac fibers of the experimental pups (Fig. 3) could be attributed to direct effect of ethanol hence oedema and fatty degeration of auricular wall are observed in acute ethanol poisoning when fatty acids are preferentially esterified to triglycerides. The pathophysiology of FAS is generally considered to be related primarily to direct toxic effects of ethanol or one its metabolites upon fetal organ development (Ukita *et al.*, 1993).

Since spleen functions as an important lymphoid organ as well as reservoir of blood (Melvin and Willian, 1993), the presence of reactive and indistinct splenic follicles in the alcohol-exposed pups (Fig. 5) as compared to those of the control (Fig. 6) would suggest early immunoreaction to alcohol. This could lead to early exhaustion of immune response mechanism and consequently increase the susceptibility of these neonates to infections. Whether cellular immune response is responsible for the observed changes in the histology of the spleen of the prenatal alcohol exposed pups requires further investigation.

In conclusion, the observed histomorphological changes in the internal organs of alcohol exposed mice may be important in the genesis of functional disturbances that characterise FAS cases.

Table 1: Mortality Rate of Mice-pups

Parameter	prenatal alcohol exposed pups	Control pups
Total No. of pups	72	78
No. of Deaths	19	0
Mortality Rate (%)	26.39	0

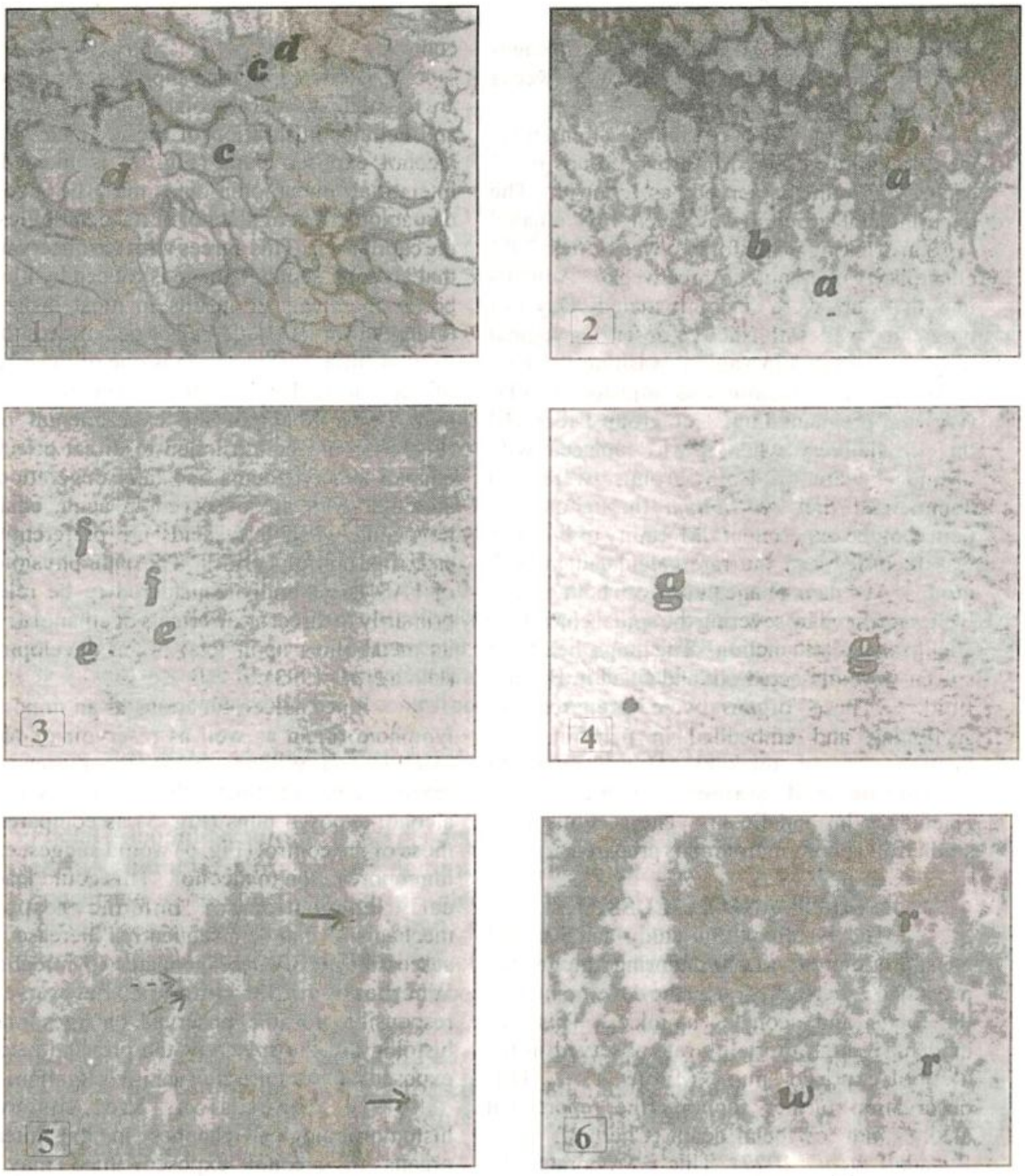

Fig. 1: Histological section of the lung of prenatal alcohol exposed mice pups X 100. Note that the alveoli are narrow (a) with thickened and cell infiltrated alveolar septae (b). X 100

Fig. 2: Histological section of the lung of control pups showing wide alveoli (c) with thin interalveolar septae (d). X 100

Fig. 3: **Histological section of myocardium of prenatal alcohol exposed pups. Observe the scanty nature of the myocardiac fibers (e) with areas of degeneration (f). X** 100

Fig. 4: Histological section of myocardium of control pups showing fully developed myocardiac fibers (g). X 100.

Fig. 5: Histological section of the spleen of prenatal alcohol exposed pups with no distinct splenic follicles. X 100.

Fig. 6: Histological section of the spleen of control pups showing distinct red (r) and white (w) pulps. X 100.

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