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Effects of Ethanol on the Uterus of Gestating Wistar Rat

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ABSTRACTS

Background: The uterus serves as protective and nutritive sac in which the conceptus develops. Any targeted assaults or intrusion to the conceptus would unlikely spare the uterus. Alcohol, a well-known toxic teratogenic agent is commonly taken as beverages, therapeutic or prophylaxis even during conception. Hence, the extent of damage to this organ with an experimental dosage of ethanol during conception in the rat was investigated in this report

Materials And Methods: 30% v/v ethanol was orally fed to an experimental group. Histological study of the uterus was done on day 15 to observe the extent of ethanol on the organ.

Results: The myomtrium was characterized by necrosis and attenuation of the muscle fibres; while in the endometrium, apoptosis of the endometrial cells were observed, with the constriction, strangulation and laceration of the blood vessels and intra-uterine haemorrhage.

Conclusion: In addition to the direct effects of teratogenic or toxic assaults on the conceptuses, the simultaneous effects of such agents on the uterus would inadvertently add to and aid the manifestations of any congenital anomalies observed.

Keywords: Ethanol, Rats, Uterus

Ethanol is a molecule that easily moves through cell membranes. It is decreasingly absorbed from the proximal portion of the small intestine, the stomach, the large intestine,the buccal and oesophageal mucosa, and rapidly equilibrates between blood and tissues, Adebisi (1995). The placenta is given credit for serving as a barrier behind which the embryo or fetus is protected from foreign chemicals. Available evidence seems to indicate that virtually all unbound chemicals in materna! plasma have access to the conceptuses across the placenta, Wilson and Fraser (1977). Many small molecules less than 600ml. Wt., and low ionic charges cross by simple diffusion, others by facilitated diffusion, action transport, pinocytosis or perhaps also by leakage. Lipophilic chemicals are known to cross the placenta and other membranes more readily than other compounds, Wilson and Fraser (1977). It now seems that the rate, as determined by size, charge, lipid solubility, affinity to complex with other chemicals and so on also play a significant role in placental permeability. Ethanol with a molecular weight of 46.07 has been shown to pass freely across the placental barrier and that its concentration in the fetus is almost as high as in the mother, Schapira (1990).

The uterus is the portion of reproductive tract that capacitates the fertilized ovum for implantation and growth of the embryo. The uterus is composed of three layers: an internal mucosa (endometrium); a middle muscular layer (myometrium) and the outer serosal covering (perimetrium).

Lately, the predominant directions of reports are on the action that produces anomalies by their effects on the placenta. Direct effects of the agents on the mothers may be as frequent as those acting directly on the foetus. However, the total dose of the chemical reaching the conceptuses is a product of interaction of many variables, some relating to maternal functional capacity, others undoubtedly reflecting the complex characteristics of the placenta. The possible interruption of utero-placenta blood flow by the chemical had also been suggested, Waddel and Marowe (1976).

Unfortunately, certainty of the specific site of action of the teratogenic agents within the maternal-placenta-foetal unit is almost non-existent. All too frequently, the naïve assumption is made that the administered agents find its way to the foetus and directly interfere with the growth and differentiation of these cells. Not only is such evidence available but also larger numbers of clues actually indicates that these chemicals do not act directly on foetal cell. For instance, as early as 1971 it was pointed out that the concentration of theteratogens, cortisone was no longer higher at its site of etertogenicity in the foetus than it was in any other foetal tissues and its site of action, and that its concentration in all the tissues were lower than in the maternal tissues, Waddel and Marlowe (1976). Comparison of maternal foetal concentation ratios of variety of chemicals with low or high tertogenic potentials revealed that this tendency was considered: that is, the potent teratogens have high foetal maternal ratios. However, this naïve assumption is untenable, that the greater the amount of chemical reaching the feotuses, the more likely the production of foetal anomalies, hence, the problem had rather now become a search for the sites within the entire maternal-placenta unit, Waddel and Marlow (1976). The present work investigates ethanol, a well-known teratogen, and a feto-toxic agent, action on the uterus at the gestational and non-gestational stages in

the Wistar rat.

MATERIALS AND METHODS

Forty (40) adult healthy Wistar rats comprising of ten (10) males and thirty (30) females were procured for the experiment from the animal holdings of the Faculty of Pharmacy, they were housed in the animal holdings of the Department of Anatomy; the room was kept tidy and well ventilated. The animals were fed on rat pellets liberally with clean drinking water provided adequately, (Sander Agro Feeds Ltd., Ibadan). The female rats were subsequently divided into two groups: A, control: pregnant-non-alcohol and B, pregnant-alcohol; each having fifteen rats respectively. The alcohol-group orally received 0.79g/kg of 30%v/v ethanol using the oesophageal tube, on gestational days 1 to 12, that is, the first and second trimesters in the animal, which coincide with the active feto-developmental period and concurrent uterine-structural adaptation stages. Ethanol dosage was calculated from its g/m equivalent weight; and 30%v/v was found tolerable to the animals following a pilot test. The control group received normal saline in lieu of ethanol. In each cage were three females and one healthy male for the purpose of mating. Confirmation of pregnancy and commencement of gestational dating followed the methods of Asling (1960).

Five rats randomly chosen from each group were sacrificed on days 12 and 15 by chloroform inhalation, and the anterior abdominal wall opened up by saggital incision, the feotuses were retrived from the gestating rats; and these together with the uterine horns were preserved in 10% formalin. Thorough histological examinations of the uterine horns were conducted following haematoxylin and eosin staining.

RESULTS

Intra-uterine hemorrhage was noticed in the ethanol treated rat uterus before retrieval and fixation. The endometrium was characterized by apoptosis of the cells and vacuoles in the connective tissues, and wide intervillous spaces with constrictions and strangulation of blood vessels in the ethanol rats, which may adversely affect nutrition and development of the foetus; while the control was characterized by gross enlargement and proliferation of the endometrium due to increased blood flow. The tissues of the myometrium are loose in orientation with the necrosis of the muscle fibres, which consequently are clearly defined due to excessive ttenuations in the ethanol rats, while hypertrophy of the myometrium was observed in the control rats (Figs. 1-2).

DISCUSSION

Ethanol in low concentration is known to depress the activity of isolated human uterine muscle. At high concentration of ethanol, sustained but reversible inhibitory effect on myometrial cells were apparent, Fuchs, and Wagner (1966). The present observations concur with results from the earlier studies of spontaneous and electrically induced isolated strips of pregnant and non-pregnant myometrium of rats; in which ethanol at high concentation may interfere with and antagonized the action of calcium at the cell membrane; in this same light, oxytocin injection given under the influence of ethanol reportedly evoked normal uterine contraction and lactation, as ethanol regulates the release of oxytocin from the neuro-hypophysis, Fuchs and Wagner (1966). More so, this experimental observations had indicated that ethanol has direct inhibitory effects on the uterine musculature; however, moderate doses of ethanol had been administered by intra-uterine infusion for the treatment of premature labour in man, since 1967 when Fuchs and co-workers described the control of undesirable uterine activities in the second half of pregnancy.

The uterus of the rat is classified as uterus duplex, Austin (1968). The Lumina of the uterine horns are completely separate and open as a paired external orifice. Partial fusion of the two horns occurs caudally in that they share a common outer longitudinal layer of myometrium. However, the circular layers merely exchange fibers. The uterine horns are 30-40nm long, the fused portion about 7 10nm. Cirucular constrictions with circular blood vessels subdivided the uterus into fetal compartments, which disappear shortly before parturition. The volume of each compartment depends on the variable number of fetuses. The strategic placement of the uterus to the fetuses inadvertently emphasize the essence of this organ, and invariably, the patho-physiologic state of the uterus tells tales on the welfare cum well being of the conceptuses; this is usually reflected in mortality or manifestation of various congenital anomalies (Alvarez, 1962; Prang and Ritter, 1995; Rudol and Stromberg, 1976).

In addition to cell damage s observed in the apoptosis of the endometrial cells, additional types of cell damage may be associated with alcohol-induced uterine damage, Spontz et al., (1994). Heavy alcohol consumption over long periods of time results in severe cell damage that leads to cell death. Cell death occurs via two distinct mechanisms: necrosis and apoptosis. Necrosis occurs when exposure to a noxious stimulus, such as alcohol, causes the loss of the cell's metabolic functions and damage to the cell membrane. In apoptosis, the cell actively participates in the cell death processes by activating a cascade of biochemical reactions that ultimately lead to cell shrinkage and fragmentation of the nucleus. When a cell undergoes apoptosis, the entire cell, including the nucleus, separates into numerous fragments (i.e, apoptotic bodies), Nanji and Hiller-Sturmhoel (1997). In any organ, both acute and chronic alcohol exposure induce

cell necrosis as well as apoptosis, and oxidative stress plays a crucial role in both processes. Oxidative injury results in the disarrangement and ultimately in the disruption of cell membranes, leading to necrotic cell death. Moreover, the oxidation of enzymes can block the metabolic processes essential for cell for cell functioning and repair. Apoptosis also can be induced indirectly by an imbalance between oxidation-reduction (Hydrogen atoms that are removed during oxidation are transferred to another molecule, which is thereby "reduced". Thus, oxidation and reduction are complementary processes, because the oxidation of one substance entails the reduction of another. This process, which moves hydrogen atoms back and forth between oxidation-reduction reactions, helps to maintain an appropriate balance between oxidation and reduction in the cell). Nicholas et al (2001). On-going work in the area of alcohol-induced apoptosis at the gonadal level will expand the knowledge in this area as germ cell apoptosis also elevated in alcohol-exposed fathers, Emmanuelle et al., (2001).

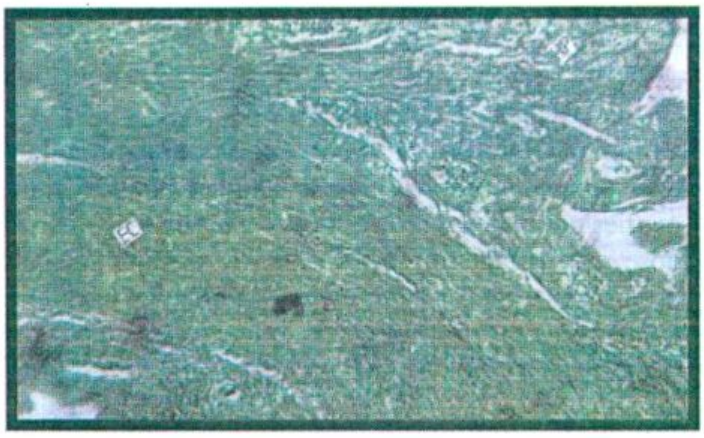

Fig. 1: Cross section of the uterus from ethanol treated pregnant rat. Note the inter-villous spaces, IS and apoptosis of the endometrium cells, AC; necrosis of the myometrium cells, NE.

Fig. 2: Cross-section of the uterus from control, non-ethanol treated pregnant rat. Note the normal hypertrophy of the myometrium, HP; proliferation of the endometrial cells, EC; and the enlarged blood vessels, BV.

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