Toxic Effects of Cigarette Smoke and Alcohol on Conceptuses of Exposed Wistar Rats

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Abstract

Introduction: Exposure to certain drugs and environmental toxins during pregnancy has been implicated in several metabolic dysfunctions affecting fetuses and neonates. This study investigated the effects of cigarette smoke and alcohol on conceptuses of the exposed female Wistar rats.

Materials and Methods: Thirty-two nulliparous female Wistar rats were used for this study. Twelve (group-1) were exposed to cigarette smoke, while 10 (group-2) were given alcohol before and during pregnancy. Another 10 pregnant Wistar rats not exposed to cigarette or alcohol were used as controls (group-3). The pregnant Wistar rats were monitored till the day of delivery and number and the body weight of the pups per litter recorded.

Results: In group-1, gestational age was prolonged significantly (p < 0.01) while the number and body weight of the pups per litter decreased significantly (p < 0.01) when compared with the controls. Meanwhile, in group-2, there was no significant change in the gestational age (p > 0.05); but the number and the body weight of the pups decreased significantly (p < 0.01) when compared with controls. Wistar rats exposed to cigarette smoke had significantly (p < 0.01) prolonged gestation when compared with those exposed to alcohol. The mean number of pups per litter were not significantly (p > 0.05) different in the two groups (alcohol and cigarette groups), but the weight of the pups exposed to alcohol was significantly (p < 0.01) lower when compared with those exposed to cigarette smoke.

Conclusion: It could be concluded that alcohol and cigarette have different levels of toxicity in the conceptuses. While our findings partly confirm previous reports on the toxic effects of alcohol and cigarette smoke on the conceptuses of the exposed Wistar rats, the prolonged gestation in the Wistar rats exposed to cigarette smoke may require further investigation.

Keywords: Cigarette Smoke; Alcohol; Pregnancy; Litters; Wistar Rats

Introduction

Cigarette smoke contains more than 4,000 chemicals including nicotine, which has its effects mediated predominantly at nicotine acetylcholine receptors widely distributed in the hypothalamus and mid-brain. The nicotine facilitates neurotransmitter release from dopaminergic, cholinergic, glutamatergic, serotonergic, and γ-aminobutyric acid-ergic nerve terminals [1]. Exposure to nicotine from cigarette smoke can change brain feeding regulation, reduce appetite and a negative energy state [2]. In addition to the altered DNA methylation and deregulated expression of micro-RNA, maternal smoke exposure affects key pathways in fetal growth and development with [3]. Various studies showed that maternal nicotine exposure during pregnancy and lactation program, the offspring to develop lung structural defect later in life with the consequent compromised lung function [4]. Hemoglobin has exceptionally high affinity for carbon monoxide content of the cigarette smoke; forms carboxyhemoglobin, fetal hypoxia and sudden infant death syndrome [5]. Also, persistent reduction of uterine blood flow, with fetal and childhood developmental disorders have been linked to cigarette smoking during pregnancy [6,7]. In Nigeria, information on the effects of cigarette smoke on pregnancy outcomes is scarce.

Alcohol is an organic chemical that is rapidly absorbed, circulates, passes through the placenta to the fetus and diffuses readily through the lipid bilayer of the cell membrane. It maintains a higher blood level with lower excretion rate in the fetus when compared with the mother [8]. It was reported that even low intakes of alcohol during early pregnancy increased the risk of spontaneous abortion and fetal death substantially [9]. The rate of stillbirth due to feto-placental dysfunction increased across alcohol categories [10]. Kuehn et al. [11] stated that after exposure to heavy alcohol during pregnancy, 80% of children had one or more abnormalities characterized by functional neurologic impairment. Polanska et al. [12] reported that consumption of alcohol during pregnancy may adversely affect children’s intelligence quotient (IQ), mental health, memory and verbal or visual performance. The chromosomal disorders observed in fetus exposed to alcohol showed phenotypic overlap with fetal alcohol spectrum disorders [13]. A study by Probyn et al. [14] showed that a liquid diet of 6% ethanol fed to pregnant dams throughout gestation caused a 3–8% reduction in fetal weight, fetal growth and brain sparing. The present study was designed to establish these disorders in Wistar rats exposed to cigarette smoke or alcohol before and during pregnancy; using the number of litters produced, period of gestation and body weight of litters immediately after delivery.

Materials and Methods

Animal Materials

Seventy-five matured Wistar rats (30 males, 45 females), of similar age ranges, each weighing 220-270 g were obtained in batches for a period of three months, from the central animal house, College of Medicine, University of Ibadan for this study. They were on feeds obtained from Ladokun Feeds Ibadan, Nigeria. The male and female Wistar rats were quarantined in separate cages for seven days for observation and acclimatization in the animal house. The female rats were randomly divided into three groups with unique identification numbers (GG 1–15 for cigarette, AL 1–15 for alcohol, CT 1–15 for controls) and were housed under standard environmental conditions. They were weighed daily and checked twice a day for any clinical signs of abnormality, morbidity and mortality until they were fully mature for mating (five to six weeks old).
Preparation of the Female Wistar Rats

A total number of 15 female Wistar rats were exposed to cigarette (London king size, containing 14.9 mg tar and 1.2 mg nicotine) smoke of four sticks per day in the exposure chamber over a period of 30 minutes per day for seven days. Since female Wistar rats go into heat (characterized by bloated vulva with an open vagina, arching her back and vibrating her ears when touched) every four to five days, mating was allowed naturally by introducing two male rats (not exposed to cigarette smoke) into each cage containing three female rats overnight for three days and the male rats separated from the female rats. Vaginal wetting or a waxy plug (mucous plug) in the vaginal of the Wistar rat was used as evidence of copulation; the date was recorded and the mated rat moved to the second compartment within the cage. The female rats were then observed for signs of pregnancy such as cessation of estrus (heat) cycle, increased appetite, weight gain, bulging out of nipples and hair loss around nipples. The first 12 Wistar rats to be pregnant (group 1) were selected for this study. The pregnant rats were further exposed to cigarette smoke till the day of delivery.

Another 15 female Wistar rats were given 15 ml of 4% alcohol daily for seven days. Since female Wistar rats go into heat (characterized by bloated vulva with an open vagina, arching her back and vibrating her ears when touched) every four to five days, mating was later allowed naturally by introducing two male rats (not exposed to alcohol) into each cage containing three female rats overnight for three days and the male rats separated from the female rats. Vaginal wetting or a waxy plug (mucous plug) in the vaginal of the Wistar rat was used as evidence of copulation; the date was recorded and the mated rat moved to the second compartment within the cage. The female rats were then observed for signs of pregnancy such as cessation of estrus (heat) cycle, increased appetite, weight gain, bulging out of nipples and hair loss around nipples. The first 10 Wistar rats to be pregnant (group 2) were selected for this study. The pregnant rats were further exposed to alcohol till the day of delivery.

A set of 15 female Wistar rats not exposed to either alcohol or cigarette smoke were allowed to mate naturally by introducing the male Wistar rats (not exposed to cigarette smoke or alcohol) into each cage containing three female rats overnight for three days and the male rats separated from the female rats. Vaginal wetting or a waxy plug (mucous plug) in the vaginal of the Wistar rat was used as evidence of copulation; the date was recorded and the mated rat moved to the second compartment within the cage. The female rats were then observed for signs of pregnancy; such as cessation of estrus (heat) cycle, increased appetite, weight gain, bulging out of nipples and hair loss around nipples. The first 10 Wistar rats to be pregnant (group 3) were selected and used as controls for groups 1 and 2.

Scoring of Pregnancy Outcomes

Immediately after delivery, the gestational age in each Wistar rat was recorded and further assessment including weight of the litters and number of litters per rat were made and recorded against the corresponding mother.

Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for windows, version 15.0 (SPSS Inc. Chicago, USA). The data were expressed as Mean ± SD. Student’s t-test was used for comparison of data in cigarette smoke / alcohol exposed Wistar rats and controls. The changes were considered significant, when p-values were less than 0.05.

Results

The effects of cigarette on pregnancy outcomes of the Wistar rats are shown in table 1. The pregnant rats exposed to cigarette had significantly prolonged gestation ($p < 0.01$) when compared with the controls. The mean number of pups per litter and their weights decreased significantly ($p < 0.01$) in the cigarette exposed group when compared with the controls. In pregnant rats on alcohol (Table 2), the number of pups per litter and their weights decreased significantly ($p < 0.01$) when compared with that of controls. But there was no significant change in the gestational age ($p > 0.05$) when compared with the controls. As shown in table 3, the effects of cigarette smoke and alcohol on the conceptsuses of the exposed Wistar rats were compared. Those Wistar rats exposed to cigarette smoke demonstrated significantly prolonged gestation ($p < 0.01$) when compared with those exposed to alcohol. The mean number of pups per litter were not significantly ($p > 0.05$) different in the two groups (alcohol and cigarette groups), but the weight of the pups exposed to alcohol decreased significantly ($p < 0.01$) when compared with those exposed to cigarette smoke.

Discussion

Gestation is a critical period during which adverse intrauterine conditions and exposure to certain chemicals or stress can influence the growth and development of the fetus. Accumulation of products of cigarette smoke such as cotinine (the metabolite of nicotine), cadmium (a heavy metal in cigarette smoke), and benzo[a]pyrene (a polynuclear hydrocarbon in cigarette smoke) have been detected in the follicular fluid of women who smoke [15–17]. The four authors reported that chemicals present in cigarette smoke can accumulate in the ovary, while other studies showed that the contents of cigarette smoke cause granulosa cell proliferation defect, increased ovarian cell apoptosis, and decreased ovarian angiogenesis [18–20]. In the present study, the reduced number of pups per litter observed is a possible consequence of ovarian insufficiency induced by the content of cigarette smoke. It could also be due to fetal hypoxia and intra-uterine death of the fertilized ova, possibly induced by carbon monoxide in the

| Table 1: Pregnancy outcomes (Mean ± SD) of Wister rats exposed to Cigarette Smoke and controls. (*Significantly different from the controls). |
|-----------------|-----------------|-----------------|-----------------|
|                  | Controls        | Cigarette       | p-value         |
| Gestational age (days) | 26.0 ± 2.5      | 37 ± 4.1        | < 0.01*         |
| Number of pups/litter | 9.0 ± 3.2       | 4.0 ± 1.4       | < 0.01*         |
| Weight of pups (g)    | 25.8 ± 5.7      | 19.1 ± 5.5      | < 0.01*         |

| Table 2: pregnancy outcomes (Mean ± SD) of Wister rats exposed to alcohol and controls. (*Significantly different from the controls). |
|-----------------|-----------------|-----------------|-----------------|
|                  | Controls        | Alcohol         | p-value         |
| Gestational age (days) | 26.0 ± 2.5      | 24.7 ± 4.8      | > 0.05*         |
| Number of pups / litters | 9.0 ± 3.2       | 3.3 ± 1.3       | < 0.01*         |
| Weight of pups (g)    | 25.8 ± 5.7      | 12.6 ± 4.3      | < 0.01*         |

| Table 3: Comparison of pregnancy outcomes (Mean ± SD) of Wistar rats exposed to cigarette smoke and alcohol. (*Significant difference between effects of cigarette smoke and alcohol). |
|-----------------|-----------------|-----------------|-----------------|
|                  | Cigarette       | Alcohol         | p-value         |
| Gestational age (days) | 37 ± 4.1       | 24.7 ± 4.8      | < 0.01*         |
| Number of pups / litter | 4.0 ± 1.4      | 3.3 ± 1.3       | > 0.05          |
| Weight of pups (g)    | 19.1 ± 3.5      | 12.6 ± 4.3      | < 0.01*         |
cigarette smoke. Our finding corroborates the report of Jin et al [21] which states that in a mother at reproductive age; 4-vinylcyclohexene diepoxide (a constituent of cigarette smoke) could cause primary ovarian insufficiency. Another study linked the impaired fertility in women smokers with combination of impaired oocyte function and viability, decreased fertilization rates, altered ovarian steroidogenesis, depleted ovarian reserves, and increased chromosomal abnormalities in oocytes [15,22]. Also in a study conducted by Roy et al. [23], was reported that nicotine reduced the efficiency of the brain and caused insufficient release of gonadotropic hormones.

The present study showed that the reduced birth weight of pups born to rats exposed to cigarette smoke. This could be associated with possible inhibitory effects of the contents of cigarette smoke on several metabolic pathways of the conceptuses of the exposed pregnant rats, since high levels of nicotine has been reported in amniotic fluid, placenta, fetal blood, and also in breast milk during lactation [6,24,25]. Luck and Nau [24] and Himes et al [26] attributed the developmental defects in the infants to exposures to higher levels of nicotine, cotinine and trans-3'-hydroxycotinine in the meconium during fetal periods. The consequences of exposure include metabolic defects and reduced body weight in the litters of the exposed rats [2,26,27]. In the report of Scott-Goodwin et al. [28], foetal growth defect and other changes observed in prenatal exposure to tobacco are associated with nicotine and carbon monoxide. In another study, Kuhnert et al. [29] reported that cadmium from cigarette smoke accumulates and binds to zinc in the placenta, lowers the level of zinc available to the fetus, causes defect in trans-placental availability of iron and low birth weight of the fetus [30]. Such exposure during pregnancy could cause decreased pregnancy-associated plasma protein A and insulin growth factors I and II levels [31], impaired organ development in the offspring and predisposition of the animal to higher risks later in life [32]. The reduced birth weight observed in our study therefore confirmed the intrauterine growth restriction reported by Robinson et al. [33], England et al. [34] and Himes et al. [26].

This study has revealed a prolonged gestation in Wistar rats exposed to cigarette smoke. Available reports on neuroimaging studies suggest that tobacco smoking causes brain atrophy in both alcoholics and neurologically normal individuals [35]. Therefore, it could be hypothesized that contents of the cigarette smoke have inhibitory effects on oxytocin metabolism and on the posterior pituitary gland of the pregnant Wistar rats used for this study. This may be justified by the report of Jo et al. [36], that there is a widely diverse nicotine acetylcholine receptors in the hypothalamus and mid-brain which bind nicotine and enhance its accumulation in the brain. This accumulation of nicotine would cause impaired brain functions in animals exposed to cigarette smoke [23,37].

Bidwell et al. [38] reported unique risk factor for specific alcohol initiation reactions during pregnancy. The significantly lower weight of pups and decreased number of pups per litter born to those Wistar rats exposed to alcohol in this study could be due to inhibitory effects of alcohol on essential metabolic pathways in the mother and fetus. Kumar [39], Thomson [40] and Strandberg-Larsen et al. [9] had hypothesized that maternal alcohol consumption during pregnancy would lead to fetal growth retardation, malformations, developmental defects, and/or spontaneous abortion. This may be possible because ethanol diffuses easily through the cell membrane lipid bilayer into the intracellular space where several metabolic activities may be hindered in the fetus. Previous workers have reported increased fetoplacental dysfunction associated with alcohol in all categories of pregnant drinkers [3,10]. Other workers reported that ethanol interferes with micronutrient metabolisms in the mother and the fetus [41]. Induces increased hepatic zinc concentration and decreased plasma zinc [42], impairs folate transport to the fetus, and lowers folate concentrations within the placenta and in the fetus [43]. Our finding agrees with the report of a study conducted by Probyn et al. [44] on pregnant dams, where exposure to a liquid diet of 6% ethanol throughout gestation caused reduction in weight, growth and brain sparing of the fetuses.

Although, a lower concentration of ethanol (15 ml of 4% alcohol) was administered into our Wistar rats, our finding disagrees with the reports of Kumar [38] and Thomson [39] who reported prolonged gestation in Wistar rats exposed to ethanol. It also contradicts the report of Andersen et al. [9] who stated that even low intakes of alcohol during early pregnancy increased the risk of spontaneous abortion. Also, this study does not agree with the report of Strandberg-Larsen et al. [9] that alcohol would cause stillbirth or miscarriage. The authors would like to suggest that interested workers should endeavor to determine the concentrations of alcohol that could cause abortion, miscarriage or prolonged gestation.

Conclusion

It could be concluded that alcohol and cigarette smoke have different levels of toxicity in the conceptuses. While our findings partly confirm previous reports on the toxic effects of alcohol and cigarette smoke on the conceptuses of the exposed Wistar rats, the prolonged gestation in the Wistar rats exposed to cigarette smoke may require further investigation.

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