Effect of prenatal exposure of alcohol in the morphology of developing rat embryo

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ABSTRACT

The objective this study was to observe the morphological changes in developing rat embryo exposed to alcohol *in utero*. Virgin female Wistar rats in experimental group (n=15) were given 20% (v/v) alcohol two weeks before mating and throughout the gestational period through oral route. The controls (n=15) were also maintained and were given the tap water. On gestational day 15 (GD_{15}) and 19 (GD_{19}), five rats from each group were sacrificed by cervical dislocation and the abdomen was incised to expose the uterine horn. The number of implantation sites and resorptions were counted and recorded. The body weight and length of the fetuses were also recorded. The litter size and body weight of the newborn were also recorded at the time of birth from the remaining dam. The incidence of resorption was higher in alcohol treated group than in control which was found to be 25% and 8.7% at days 15 and 19 respectively. The body weight and length of fetuses were found to be decreased and was significant at GD_{15} ($p<0.001$ for weight and $p<0.05$ for length). Similarly, the litter size and body weight of newborn were also found to be decreased significantly ($p<0.05$ for litter size and $p< 0.01$ for body weight). The present study shows that the maternal consumption of alcohol during pregnancy has adverse effect on fetal viability and development of growing embryo.

Keywords: Wistar rat, alcohol, embryo, pregnancy.

INTRODUCTION

The potential teratogenic effects of alcohol have been suspected for centuries.[1] However, it was not until the work of Lemoine *et al* in 1968 and Jones *et al* in 1973 that a distinct dysmorphic features associated with maternal gestational alcoholism was described in medical literatures.[2,3] In fact, it was Jones *et al* who coined the term fetal alcohol syndrome (FAS) to describe the various morphological, physiological and behavioural abnormalities in the newborn associated with maternal consumption of alcohol during pregnancy.[3] Since then, several studies related with FAS have been published in medical literatures of many countries. In addition to clinical and epidemiological studies, many investigators have attempted to observe the effect of alcohol in animals. They provide an insight into the effects of alcohol on the developing embryos. Malformations, intrauterine death, growth deficiency, CNS abnormalities and behavioural deficits have all been demonstrated in laboratory animals exposed to alcohol *in utero*.[4-7]

In our country, the literatures lack information regarding the clinical report of fetal alcohol syndrome and the experimental research studying the effects of maternal alcohol consumption on developing fetuses. Hence, the present study was conducted to observe the direct effect of maternal alcohol consumption during pregnancy on the development of embryos by using rat as an experimental model.

METHODS AND MATERIALS

Adult virgin female albino rats (n=30) with initial body weight ranging from 150-200 grams were used. All animals were acclimatized to laboratory condition for one week before treatment. All animals were maintained in a well ventilated room under controlled temperature (25 ± 5^0C) and natural light and dark cycle. Animals were kept in groups consisting of five rats per cage made up of propylene (size= 40 cm ´ 25 cm ´ 15cm). All the animals were fed standard pellet diet (produced in Dhulabari, Jhapa) and Bengal gram. They were given tap water *ad libitum*.

EXPERIMENTAL SET UP

Animals were randomly divided into two groups, each group consisting of fifteen rats. The first group was given tap water and designated as control while the other group was given 20% v/v ethanol solution through oral route one week before mating till the weaning of their offspring and was designated as experimental group. The rats in the experimental group were treated with a 5% and 10% v/v ethanol solution for two consecutive weeks before treatment with 20% v/v ethanol solution.

Female albino rats from both groups during normal estrous cycle were kept with healthy males of the same strain in the evening. The presence of vaginal plug and sperm in vaginal smear the following morning confirmed the successful mating and the day was considered as day zero of gestation (GD_0). Pregnant rats were kept in individual cage with their provided diet available. At

8:00 am on gestational day 15 (GD_{15}) and gestational day 19 (GD_{19}) five rats from each group were sacrificed by cervical dislocation. The abdomen was incised immediately and the uterine horns were exposed. The total number of implantation sites and resorptions were counted and recorded. Further, fetus were examined for developmental anomalies. The weight and size (body length) of the fetus were also recorded. Beginning on day 20 of gestation the remaining dams of control as well as experimental groups were routinely monitored until delivery to determine the litter size. The body weight of rat pups were recorded at the time of birth and was also looked for other gross visible developmental anomalies.

RESULTS

The number of implantation and resorption that occurred on gestational days 15 and 19 in control and experimental groups clearly indicates the adverse effect of alcohol on fetal viability. Table I shows 80% and 40% rats with resorption on GD_{15} and GD_{19} respectively among the experimental group. The number of total implantations did not differ significantly for the control and experimental groups. However, the incidence of resorption was higher in alcohol treated rats which was found to be 25% and 8.7% at days 15 and 19 respectively. Beside, on GD_{19} a small area of hemosiderin representing the resorption was observed at the original site of implantation. In one alcohol treated dam on day 15 of pregnancy, all implantations were resorbed whereas no resorption was observed in control group.

Table-1: Effect of alcohol on fetal viability

(GD Gestational day	Groups	PR	RR (%)	TI(site /dam)	TR (%)
15	Control	5	0	31	0
	Experimental	5	4 (80.0%)	28	7 (25.0%)
19	Control	5	0	28	0
	Experimental	5	2 (40.0%)	23	2 (8.7%)

GD= Gestational Day, PR= Pregnant Rat, RR= Rats with Resorption,

TI= Total Implantaion, TR= Total Resorption

Table-2: Body weight and length of fetus on gestational days 15 and 19

Gestational day	Groups	Body weight (gm) (mean ± sd)	Body length (mm) (mean ± sd)
Day 15	Control	0.21 ± 0.017***	11.42 ± 0.90*
	Experimental	0.16 ± 0.016***	10.64 ± 0.51*
Day 19	Control	1.51 ± 0.123	24.36 ± 1.56
_	Experimental	1.45 ± 0.206	24.00 ± 1.35

****pd<0.001, *pd<0.05*

The body weight and length of fetus of control and experimental groups at GD_{15} and GD_{19} is shown in Table-2. The body weight and length of fetus at GD_{15} were 0.21 ± 0.017 gm and 11.42 ± 0.90 mm for control whereas the values were 0.16 ± 0.016 gm and 10.64 ± 0.51 mm for experimental group respectively. Similarly the body weight and length of fetus at day 19 were 1.51 ± 0.123 gm and 24.36 ± 1.56 mm for control and 1.45 ± 0.206 gm and 24.00 ± 1.35 mm for experimental group respectively. Although mean weight and length of fetuses were found to be decreased in experimental group at days 15 and 19, the decrease in body weight and length were found to be significant (pd"0.001 for weight, pd"0.05 for length) at day 15 when compared with that of control group.

Table-3: Effect of prenatal exposure to alcohol on litter size and body weight of pups at birth

Observations	Control	Experimental
Litter size per dam	6.2 ± 0.84*	4.4 ± 0.55*
Body weight at birth (gm)	4.71 ± 0.39**	4.39 ± 0.12**

****pd<0.01, *pd<0.05*

Table-3 shows the intrauterine effect of alcohol on litter size and fetal body weight at birth. The litter size i.e. number of rat pups from alcohol treated dam was smaller (about 4) than that from control group (about 6). The difference was statistically significant (pd"0.05). Similarly, at birth the body weight of rat pups born to alcohol treated dam was found to be 4.39 ± 0.12 gm which is significantly lower p £ 0.01 when compared with the body weight of rat pups (4.71 ± 0.39 gm) born to alcohol non-treated rats.

DISCUSSION

Present study has demonstrated that chronic ethanol consumption during pregnancy is embryotoxic and/or embryolethal. The increased frequency of resorptions clearly indicates the adverse effect of prenatal exposure of alcohol on fetal viability. This result agrees with other studies, when alcohol was administered before and throughout pregnancy. The decreased number of resorptions observed on day 19 as compared with day 15 may be due to the fact that by day 19 the only vestige would be a small area of haemosiderin at the original site of resorption as suggested by Sanchis *et al* also in their study.[8] Further, the small litter size in experimental group as compared with that of control also suggests the detrimental effect of alcohol on embryos *in-utero* indicating that ethanol can play an important role in embryolethality. Chronic ethanol intake at the time of conception and during pregnancy in human has also been associated with increased risk of spontaneous

abortion.[9,10] Although the exact causes are still unclear, it is known that alcohol interferes with the normal function of the reproductive system e. g. alcohol alters the oestrous cyclicity and levels of prolactin and luteinising hormone prevents conception and implantation.[11-15]

The prenatal exposure to alcohol causes intra-uterine growth retardation. In this study, the body weight and length of fetus at gestational days 15 and 19 were also found to be decreased in experimental group as compared to those of control. Besides, the body weight of rat pups at birth was also significantly decreased in experimental group as compared to the controls. It has been demonstrated that the children born to alcoholic mothers are of small size (weight and/or height) for given gestational age.[2,3,16,17] The mechanism by which alcohol affects the fetal growth is still unclear. It is well known that fetal development depends on proper function of placenta which regulates the transfer of various substances, e.g. amino acids from mother to fetus, that is essential for normal growth, and maintenance of developing fetus. It is reported that alcohol inhibits the transport of glucose, vitamin B6 and amino acids across the placental tissue by altering its cytoarchitecture, transport mechanism and protein synthesis leading to malnutrition both in human and in animals.[18-20] Besides, hypoxia and free radical toxicity are also considered to affect the fetal development *in-utero*.[21,22]

In the present study, no appreciable malformation and internal anomalies were observed in the offspring of alcohol treated rats. It must be realized that the severity of effects in the offspring is closely related to the chronicity of mother's alcoholism.[23] Sanchis et al, in preliminary study of their work in which female rats were subjected to long-term alcohol treatment (approximately 15 weeks), had observed congenital malformations in the majority of offspring (60%).[8] Further, a number of epidemiological and experimental studies have already proven that alcohol is capable of producing congenital malformations in developing embryos.[4-7] Therefore, keeping in mind the possible teratogenic effect of alcohol on developing fetus, it is suggested that women should avoid taking alcohol during pregnancy.

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