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Adverse Effects of Alcohol on Pregnancy and Its Outcome – A Study on Mice

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Abstract: ***Background: For centuries, social studies all over the world have shown that maternal drinking during pregnancy can have serious adverse effects on the health of the newborn. Alcohol-related damage to the fetus has been linked with every form of drinking pattern, from heavy drinking to intermittent as well as moderate social drinking i.e. one or two drinks daily most days during pregnancy; and from very infrequent, but relatively heavy, drinking to one single drinking binge prior to pregnancy recognition. Objective: The present work was undertaken to study: the possible teratogenic effects of ethanol exposure on developing mice embryos; its dose effect relationship and the time effect relationship. Material & Method: 36 Pregnant mice were administered alcohol orally with a syringe and cannula. The dose effect was studied by choosing two doses of alcohol; and the time effect relationship with a particular dose is given during total gestational period (0-18 days) or during major organogenesis period (6-15 days). The 189 fetuses, delivered by caesarean section, were examined for gross anomalies. Result & Conclusion: It was concluded that the resorptions, intrauterine growth retardation, limb anomalies like ectrodactyly, wrist drop, club foot and curly tail were the major adverse effects of ethanol.***

Keywords: alcohol, curly tail, ethanol, Fetal alcohol syndrome, limb anomaly, Teratogen.

1. Introduction

In recent years, it has been observed that patterns of alcohol use are changing, with more and more teenagers consuming alcohol regularly. knowledge of alcohol-related birth defects dates back to times and there were even laws prohibiting the use of alcohol by newly married couples in order to prevent conception during intoxication [1] The teratogenic effects of ethanol on human fetuses were first reported in 1968, describing a common pattern of birth defects in children born to alcoholic mothers in France.[2] A wide variety of variable pregnancy outcome have been reported after controlled human studies on this aspect. In 1973, Jones & Smith[3] described specific in infants of alcoholic mothers and named the symptom complex as Foetal Alcohol syndrome (FAS).

There has been no teratogenic agent yet studied in man which has shown a clear threshold effect, i.e. where the substance could be considered safe at a particular level, beyond which its teratogenic effect begins to take hold, and alcohol is no exception [4]. Thus the present study was envisaged to elicit in mice the possible teratogenic effect of alcoholic exposure .Dose effect relationship by choosing two doses of alcohol and the time effect relationship when a particular dose is given during (i) total gestational period (ii) during major organogenesis period was also studied.

2. Material and Methods

The study was done on sexually mature Swiss Albino mice. After mating, 36 pregnant mice were divided into three groups with 12 mice in each as follows-

Group I (control group): This group was kept under similar and identical experimental conditions as treatment groups. Each mouse received 0.5 ml of distilled water per orally on the scheduled dosage days.

Group II (Low dose treatment group): Each pregnant mice of this group was administered 25% of ethanol v/v at the dose level of 2.9g/kg body weight/day /orally.

Group III (High dose treatment group): Each pregnant mice of the group was administered 25% of ethanol v/v at the dose level of 5.8 g/kg body weight/day/orally.

All these groups were further divided into A and B subgroups with 6 mice each. Subgroup- A was administered the distilled water or ethanol throughout the gestation i.e. 0-18 days, while Subgroup B was administered the dose during the period of major organogenesis i.e. 6-15 days). The dosage was administered orally with the help of a syringe and cannula. During the entire period of experiment the mice were fed on regular animal diet as pellets. The day of vaginal plug was taken as day 0. Fetuses were delivered by caesarean section on day 19^{th} of gestation.

Foetuses were examined for:
1. Mean foetal weight & length
2. Gross anomalies

The uteri of pregnant mice which showed total resorptions were subjected to histological examination to confirm the findings.

3. Results

During the entire period of experiment the general health of 36 dams of all the groups was normal, and food and water intake was satisfactory. All the dams reacted to the ethanol administration: got comatose at higher dose level and exhibited locomotor ataxia at lower dose level. 3 dams died during study one each from subgroup IIA, IIIA,III B. The dam of Subgroup II-A (low dose treatment group) died on 10^{th} day. The 2 dams of Subgroups III-A on and III-B (high dose treatment group) died on day 16^{th} & 17^{th} respectively. Maternal Mortality was higher in high dose group.

All the dams showed a steady gain in body weight, although dams of II-A and III-A subgroups, which were given ethanol for the whole pregnancy period, showed least gain- 15.90g & 12.57g respectively. The gain in body weight of control dams was highest -22.00g.

The resorption rate was lowest in control group while the number of resorptions were higher in IIA & IIIA subgroups. Two of the pregnant mice in IIA and four of the pregnant mice in IIIA showed total resorption.(fig 1-3) Histological study conducted on the uteri of group II-A and III-A showing pinpoint black resorptions on gross examination revealed the ruptured embryo and haemorrhage in Placenta. (Fig. 4)

The mean litter size was highest in IB (8.51) and lowest in IIIA (1.17).A total 189 foetuses (184 live and 5 dead) were obtained. In the alcohol treated groups the average foetal weight and length were found to be lower than the control group. (Table 1)

Table 1: Summary of observations in the study

GROUP	Control Group		Low-Dose Group		High Dose Group	
Treatment material	Distilled water		25% Ethanol		25% Ethanol	
Dose (per day)	0.5 ml		2.9 g/kg		5.8 g/kg	
SUB-GROUPS	I-A	1-B	II-A	II-B	III-A	III-B
Day of gestation when treated	0-18	6-15	0-18	6-15	0-18	6-15
No. of pregnant mice	6	6	6	6	6	6
Av. gain in body wt. during pregnancy (gm)	22	21.5	15.9	18.3	12.5	20.3
No. of resorptions (Mean)	0.5	0.5	3.4	1.5	6.67	2.67
Mean litter size	5.8	8.51	4.6	6.17	3.5	5.17
Total no. of live fetus	35	51	23	37	7	31
Total no. of dead foetus	1	0	0	1	1	2
Mean foetal weight (gm)	1.20	1.25	1.02	1.16	1.11	1.00
Mean foetal length (cm)	2.22	1.92	1.83	1.78	1.76	1.66
Gross anomalies (%)	8.3	9.8	30.4	21.0	25.0	33.3

On gross examination the the morphological anomalies were seen maximum in Group III B (33.3%) followed by Group II A (30.43%). (Table 2)The fetuses showed forelimb anomalies as wrist drop, ectrodactyly, syndactyly and hind limb anomaly as club foot. Curled tail was a common finding in the treated group. (Fig.5-8)

Table 2: Details of the Gross morphological anomalies & Skeletal Anomalies

Group/ Subgroup	*Fetus*	*Gross Morphological anomalies*	
	189	Affected	Description
IA	36	3 (8.33%)	Dome shaped head,syndactyly
IB	51	5(9.80%)	Wrist drop, everted claw
IIA	23	7(30.43%)	Wrist drop, everted claw, curled tail
IIB	38	8(21.055%)	Dome shaped head, depression in occipital region, wrist drop, hyperextended forelimb,curled tail
IIIA	8	2(25%)	Wrist drop, curled tail, club foot
IIIB	33	12(36.36%)	Dome shaped head, forelimb-wrist drop, syndactyly, ectrodactyly, partial ectrodactyly, curled tail, hindlimb- syndactyly

4. Discussion

The present study was undertaken on the mice, which has been previously reported to be the suitable animal model for foetal alcohol syndrome [5]. Alcohol is a low molecular substance and is therefore quite capable of crossing the placental barrier and entering the fetus, causing the level of alcohol in the fetus to be approximate to that of the mother [6]. It has been observed in the study that there was significantly raised incidence of resorptions in group II-A and III-A, 4 mice (IIIA & IIA group) showed total resorption. It has been stated that exposure to alcohol and other 'spindle-active' substances that have a similar action on the meiotic spindle apparatus during the menstrual cycle before conception can induce chromosome segregation errors in the ovulated oocyte. The successful fertilization of such eggs consequently results in the production of aneuploid embryos, which have a very high chance of being spontaneously aborted during the first trimester of pregnancy. Those relatively few aneuploid conceptuses that survive to term invariably show moderate to severe degrees of mental retardation, craniofacial and other abnormalities, as well as having a significantly reduced life expectancy. [7]

Histological observations on the uteri of group II-A and III-A showing the ruptured embryo and haemorrhage in Placenta suggested early abortifacient effect of alcohol and could be a possible cause of missed abortions in humans. The references for such histological study could not be found. However similar embryotoxic effects have also been reported in past. [7,8,9,10,11].

In the study fetuses of group II and III had lesser birthweight than the controlled group. In the study the fetuses which survived in the alcohol treated groups showed the average foetal weight and length lower than the control group, implying the adverse effect of ethanol on the intrauterine growth of foetuses. [2,3,12-16]

The anomalies were found to be more qualitatively and quantitatively among the fetuses of high dose group than the lower dose and control groups. The incidence of anomalies increased with the increase in duration of treatment i.e. it was more among the fetuses exposed to ethanol during total intrauterine life which included Curled tail, and anomalies of limbs as syndactyly, ectrodactyly, arthrogryposis, club hand and foot .

Multiple limb anomalies such as syndactyly, ectrodactyly and clubfoot have also been reported in past studies[17]. Growth retardation as found in our study and the dose response effect of alcohol intake has also been observed by other researchers[5, 9].

It has been studied that under normal embryonic development process by the time the mouse embryo has about 24 pairs of somites (late day 10 of gestation). the fore limb buds appear at the level of somites 5 to 12; and by the end of day 12 the hindlimb can be observed. The basic development of both forelimbs and hindlimbs is completed by day 16 of development. At birth, all five digits (of each limb) are formed .[17] Limb anomalies as found in the present study can be explained on this basis. The dosage days in sub group A :0-18 days & Subgroup B :6-15 days predisposed for the limb anomalies in the fetuses. Variable musculoskeletal and limb defects were found in approximately 40% of cases, ranging in severity from minor problems such as contractures of the finger joints to more severe lesions, such as congenital hip dislocations and thoracic cage abnormalities [18].

In one study in past[19] 49% of the fetuses were resorbed or dead and 46% of the survivors showed forelimb ectrodactyly. Ectrodactyly induced by ethanol was primarily of the forelimb and exclusively postaxial. In another study on mice ,out of 102 treated fetuses, 44 had limb defects affecting the distal ends of forelimbs.[11] In the present study as well 4 mice (IIIA & IIA group) showed total resorptions. The survivors showed curly tail, forelimb anomalies as ectrodactyly, syndactyly, club hands. Many of the fetuses, which were exposed to higher dose of alcohol throughout the intrauterine life, exhibited multiple anomalies, thus, suggesting that alcohol acts as general embryotoxin.

The failure of closure of the spinal neural tube, which leads to spina bifida in the mouse, has been traced back to a tissue-specific defect of cell proliferation in the tail bud of the E9.5 embryo. This cell proliferation defect results in a growth imbalance in the caudal region that generates ventral curvature of the body axis. Neurulation movements are opposed, leading to delayed neuropore closure and spina bifida, or tail defects. [21] The higher incidence of curled tail in the present study signifies the neural tube defects resulting as an adverse effect of alcohol.

It has been demonstrated repeatedly that high alcohol consumption during pregnancy may seriously affect the developing embryo. The higher the blood alcohol of the mother, the greater the damage to the developing fetus[9,11,19,21,22] . The severity of the malformations ranges from FAS, to minor effects, such as low birth weight, Intra Uterine Growth Retardation (IUGR), a slight reduction in IQ of the infants and increased rate of congenital anomalies [3,5,23-25]. Similarly the anomalies were found higher in high dose group in the present study.

The results found in the mouse model in this study can be correlated with the adverse effects of alcohol as observed in humans. In humans by the end of the 36th day, often long before the woman even realises that she is pregnant, the neural tube is clearly present and open, and most of the rudimentary organs have already been formed, such as limbs, heart, brain, eyes, mouth, digestive tract. It is therefore obvious that if a teratogenic substance such as alcohol is consumed during this most critical period of rapid growth of cell development and organ formation, this can result in various forms of malformation in the newborn, such as defective heart, musculoskeletal abnormalities, mental handicap etc., without any specific outward signs of FAS.

5. Conclusion

It can be concluded that the ethanol is an embryotoxin. It may result in resorptions and intrauterine growth retardation. The dose effect relationship and time effect relationship was established conveying that the amount of alcohol ingested, the length of period consuming alcohol and the developmental stage of the foetus at exposure mediate the effects of ethanol intake on the developing foetus. Alcohol consumption can be an underlying cause of infertility and missed abortions in females. The intrauterine growth retardation, morphological limb anomalies,curled tail implying neural tube defect are the adverse effect of alcohol on the pregnancy outcome. Alcohol is a poison at all levels, and therefore no totally safe level of alcohol use during pregnancy can be established.

6. Conflict of Interest

None

7. Acknowledgement

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Legends of Figures

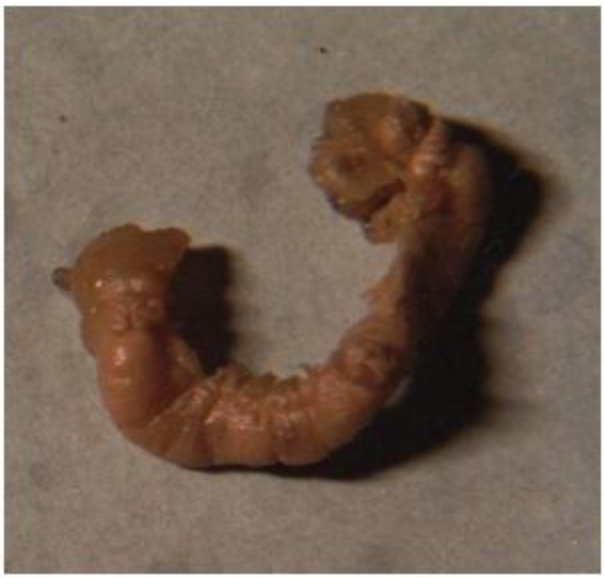

Figure 1: Uteri with late resorptions

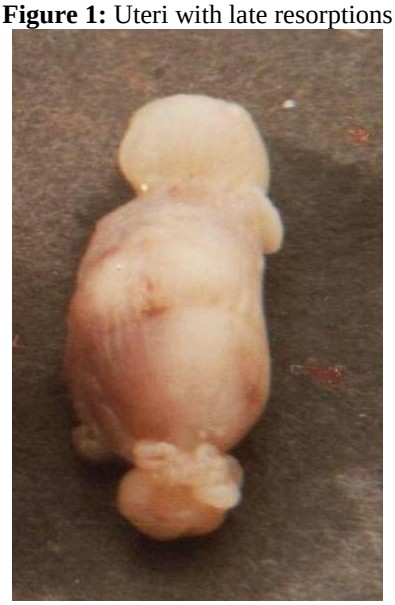

Figure 2: Resorbed fetus

Figure 3: Uterus with pinpoint resorption

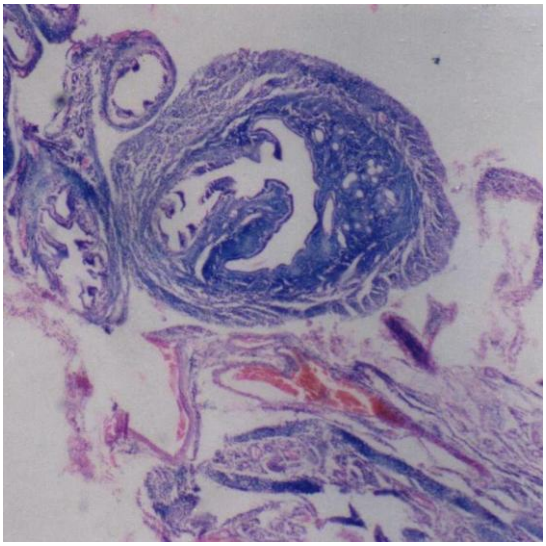

Figure 4: Histological examination of Uteri showing rupture of embryoblast & Placental Haemorrhage signifying early abortion.

Figure 5: Fetus with IUGR, Dome shaped head, Arthrogryposis of shoulder joint,elbow joint, wrist joint, knee joint and curly tail

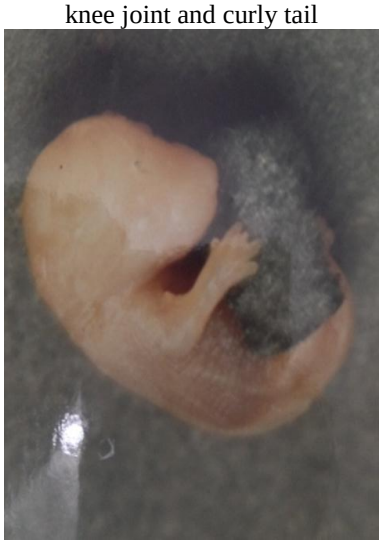

Figure 6: Fetus with Partial Ectrodactyly of middle finger, Syndactyly with2 & 3 digits

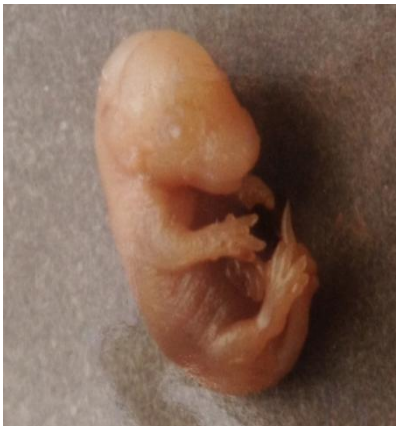

Figure 7: Fetus with Ectrodactyly of middle finger, Syndactyly with2 , 3 digits & 3, 4 digits of hind limb

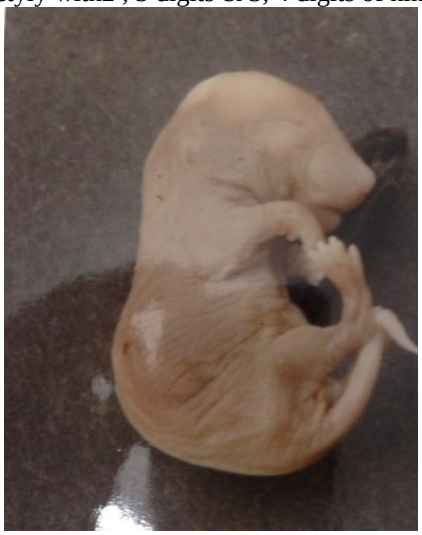

Figure 8: Fetus with wrist drop and curly tail.