

Study of Prenatal Ethanol Exposure on Postnatal Intestinal Development, Milk Consumption and Fertility Indices in Intoxicated Rats-Born Offspring

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Abstract: Background: Exposure to ethanol either during the pregnancy or lactation period induces marked alterations in growth and development of offspring's collectively known as fetal alcohol syndrome (FAS), however, its effects on the postnatal intestinal development, milk consumption and fertility indices and are not adequately investigated. We evaluated, intestinal status, milk consumption of litter/the offspring born and several indices such as fertility, viable gestation and the survival index, hence this complementary study was performed regarding pregnancy outcome. Materials and Methods: Pregnant female rats beginning at day 1 of gestation were fed ethanol daily during the entire gestation or lactation separately. To study the effect of chronic alcoholism during gestation or lactation separately. At birth, Control newborns were cross-fostered to Ethanol-exposed Mother rats (EM), and the offspring issued from the ethanol exposed mothers were cross-fostered to Control mother rats (CM). Hence, there were 3 experimental groups of offspring formed: (A) Control offspring receiving No treatment (CO); (B) Offspring exposed to Ethanol only during Gestation (GO); and (C) Offspring exposed to Ethanol only during Lactation (LO). All the results were compared with offspring Pair-fed groups (PFO) born of the Pair-fed Mother rats (PFM). Results: Results of current study found no significant difference in food intake and body weight of unexposed and ethanol exposed pregnant female rat. Offspring exposed to ethanol only during lactation show a significantly lower body weight compared with the control groups (CO, PFO) and the offspring exposed to ethanol only during gestation at Day 21 of lactation ($P < 0.001$). Milk consumption was significantly lower for alcohol-administered groups (GO and LO) compared with control groups (CO, PFO; $P < 0.001$). In-addition there was a slight insignificant reduction, for number of litter size in the EM and PFO groups compared with the CM. In the PFM group too there was an insignificant slight reduction, of fertility index and that of viable gestation. The levels of testosterone were significantly decreased in EM and in the PFD ($P < 0.001$). Conclusion: The findings of our study suggested that ethanol exposure either during the pregnancy or lactation period induces marked postnatal development specifically seen in certain intestinal status, milk consumption and fertility indices of ethanol intoxicated rats in utero.

Keywords: Prenatal ethanol exposure, Postnatal Intestinal development, Fetal alcohol syndrome, Fertility indices.

1. Introduction

Pregnant alcoholic female risk the health of their offspring in multiple ways: A. Exposure to alcohol during gestation may lead to fetal alcohol syndrome (FAS) or fetal alcohol effects; B. the physical consequences of alcoholism in the female (e.g., falls or malnutrition) may independently affect the developing fetus; C. genetic vulnerability to alcoholism in the fetus may increase the effects of prenatal exposure; and D. the lifestyle of an alcoholic parent may lead to negative consequences for the fetus, the pregnancy, and the developing offspring [1]. Chronic ethanol ingestion during gestation is responsible for (FAS), which is associated with general growth retardation and multiple birth defects in offspring [2]. Major consequences of FAS are facial deformities, central nervous system dysfunctions and nonspecific malfunctions [3-4]. Earlier studies in experimental animals have demonstrated that prenatal ethanol exposure leads to gastro-intestinal tract damage, also retards intestinal growth [5] modifies the development of intestinal brush border enzymes [6] and impairs the absorption of glucose, amino acids and macromolecules [7-8] during postnatal development in rats. Traves et al [9] described that ethanol metabolism in developing fetus is

negligible, thus intragastric ethanol levels are several times higher than in other fetal compartments. Such high levels of ethanol could induce structural and functional deformities during the period of intense cell proliferation and differentiation. Most studies on prenatal alcoholism have focused on the end of gestation as a model of fetal alcohol syndrome [10]. However, a fostering / cross-fostering model during lactation (on Day 2 after birth) proposed in this study allowed us to determine the postnatal developments in utero or in the suckling period in offspring, separately. A complementary study was performed on the pregnancy outcome. We evaluated testosterone values, the offspring born/litter and several indices such as fertility, viable gestations and survival index. In the present study, postnatal development of intestinal status was investigated in rat exposed to ethanol during gestation or lactation along with fertility indices and milk consumption.

2. Material and Methods

2.1 Animals

Wistar strain albino rats (100-120 g body weight) were used. Animals were maintained under an automatically controlled

temperature (22–23°C) and a 12-h light–dark cycle (9:00–12:00 h). The female animals were kept for mating with male rats to obtain the first generation offspring. The first day of gestation (day 1) was checked by examining the vaginal smears under microscope as described by Baker [11]. Pregnant rats were housed individually in plastic cages. Usually 4–6 female rats were used in each group. Pregnant female rats were randomized into three mother groups during entire gestation or lactation period as: Control mother rats (CM), Ethanol exposed mother rats (EM), and pair-fed mother rats (PFM). CM was given water and basic diet *ad libitum* during the entire experimental period. EM: ethanol and basic diet *ad libitum*. PFM: water and isocaloric diet with the diet of ethanol rats. The day of parturition was designated as Day 1 of lactation, Day 30 being the end of the lactation period. The offspring number was reduced to 6–8 per mother from the time of parturition. The experiments were performed on the offspring of three groups at Day 30 postpartum. During the suckling period, the pups had free access to the nipples. At birth (second day postpartum because the 1 day of lactation period the mother rats rejected to the new infants), the offspring were divided into four experimental groups: CO: Control new-born, born and breastfed by their control unexposed mother rats. LO: Control new-borns cross-fostered to ethanol exposed mother rats, offspring exposed to ethanol only during lactation. PFO: Pair-fed new-borns, born and breastfed by their pair fed mother rats. Upon delivery, the neonates were kept with their natural mothers and were sacrificed at different days of postnatal age.

2.2 Ethanol treatment

Ethanol was administered in tap water by a previously described method [12]. Briefly, alcohol treated rats were given 5% ethanol (v/v) *ad libitum* for 1 week (5.5 ± 0.2 g ethanol/kg/day), 10% ethanol during the second (7.8 ± 0.4 g ethanol/kg/day), 15% during the third (8.9 ± 0.4 g ethanol/kg/day), and 20% ethanol during the fourth week (16.6 ± 2.1 g ethanol/kg/day). A consumption of 20% was maintained for 4 additional weeks. The total calories at the end of the experiment was similar in the different groups (CM: 163.10 ± 11.44 ; EM: 150.11 ± 1.82 ; PFM: 155.97 ± 10.83). Ethanol-treated rats were mated. The presence of sperm in the vaginal smear the following morning denoted Day 1 of pregnancy. Pregnant females were replaced individually in their cages and assigned again to 20% ethanol as the sole source of liquid with food *ad libitum*, during the pregnancy and lactation periods. Offspring received ethanol through the maternal milk route.

2.3 Diets

Diets were prepared, which details the known nutrient requirements for most of the common laboratory animals (g/kg of diet): Casein: 200; Sucrose granulated: 510; Cornstarch: 140; Fiber, cellulose: 50; Corn oil: 50; AIN-76 mineral mix: 35; AIN-76 vitamin mix: 10; Choline bitartrato: 2; DL-methionine: 3; Zinc: 0.029. Diet ingredients, including mineral and vitamin components, were mixed and homogenized (in the department of biochemistry laboratory Basic Medical Science Building, Panjab University, Chandigarh) in a double-cone blender

(Rest Haan, Germany). Animals were maintained on commercial rat diet. The diet offered to animals as pellets.

2.4 Gestational parameters

A complementary study was performed to understand the pregnancy outcome. We evaluated testosterone values, the offspring born and other several indices. The serum testosterone was determined by fully automated immunoassay system-Vidas make of France using ELFA (Enzyme Linked Fluorescence Assay) technology. The results of indices are expressed as a percentage: Fertility index (FI): number of viable gestation/number of animals. Viable gestation index (GI): number of successful gestation/number of animals. Survival index: number of newborn alive / total number of newborn.

2.5 Milk samples

In order to obtain the maximum amount of milk without modifying the physiological conditions of the subjects with anesthetics, on Day 30 of lactation 3 h after removing the offspring from their mothers, Mother rats were sacrificed by decapitation and milk samples were immediately collected. Milk was obtained by gently massaging the area around each of the 12 mammary glands and then pressing upward from the base of the gland toward the nipple.

2.6 Milk consumption

The amount of milk consumed was estimated by subtracting the weight of the offspring obtained just prior to returning them to the mother rats from the weight at the end of 30 min of suckling.

2.7 Statistical methods

Mean differences were studied by using Student's test or analyses of variance (ANOVA). Statistical significance established at $P < 0.05$. When ANOVA resulted in differences, multiple comparisons between means were studied using Tukey–Kramer test.

2.8 Ethical clearance

Ethical clearance: The Department of Research and Development's Ethical Committee and Review Board of Dr. A.P.J Abdul Kalam University, Indore, India fully authorized the protocol. The experimental protocol was approved by the Institute's Ethical Committee. Experiments on animals were conducted in accordance with the Guidelines for use of Laboratory Animals in Medical College, Indian Council of Medical Research (ICMR), New Delhi, India.

3. Results

3.1 Body weight

The gestation period was lengthened by a day, from 21.5 ± 0.7 in controls to 22.5 ± 0.9 days in ethanol-exposed group. There was no difference in food intake and body weight of pregnant female rats in control and ethanol exposed groups. Ethanol consumption during pregnancy didn't cause

alterations in body weight of offspring at birth. Body weight at 30 days was lower in offspring fed with ethanol- exposed mothers than in CO. LO offspring show a significantly lower body weight compared with the control groups (CO, PFO) and the GO at Day 30 of lactation ($P < 0.001$). (Table 1)

Milk consumption was significantly lower for alcohol-administered groups (GO and LO) compared with control groups (CO, PFO; $P < 0.001$). There were no significant differences between the two groups of offspring exposed to ethanol. Results are reported in Table 1.

3.2 Milk consumption

Table 1: Effects of pre or postnatal ethanol consumption on bodyweight and milk consumption in the offspring rats

Offspring (n=15)	CO	EO	PFO	
Bodyweight at birth (g)	6.77±0.26	6.11±0.15	6.56±0.22	
Offspring (n=15)	CO	EO	PFO	
Bodyweight at Day 4 after birth (g)	12.75±0.20	12.15±0.12	12.56±0.21	
Offspring (n=15)	CO	EO	PFO	
Bodyweight at Day 8 after birth (g)	24.76±0.22	24.108±0.13	24.55±0.21	
Offspring (n=15)	CO	EO	PFO	
Bodyweight at Day 21 after birth (g)	42.90±1.22	38.10±1.28	27.63±1.23	
Offspring (n=15)	CO	GO	LO	PFO
Bodyweight at Day 30 after birth Days postpartum (g)	68.00±1.90 $P < 0.001$	63.10±0.10 *** $P < 0.001$ ° $P < 0.05$	54.61±7.07 *** $P < 0.001$ ³ $P < 0.05$	61.68±1.24 *** $P < 0.001$ ¹ $P < 0.001$
Milk consumption (g)	1.52±0.01	1.19±0.06 *** $P < 0.001$	1.017±0.05 *** $P < 0.001$ ³ $P < 0.05$	1.3±0.03 *** $P < 0.001$ ¹ $P < 0.001$

The results are expressed as mean ± SEM. Differences are analyzed by a multifactorial analysis of variance (ANOVA) followed by the Tukey's test. n' indicate the number of animals in each group. GO versus CO: *** $P < 0.001$; LO versus CO: *** $P < 0.001$; PFO versus CO: *** $P < 0.001$.

GO versus LO: ³ $P < 0.05$; PFO versus LO: ¹ $P < 0.001$. GO versus PFO: ° $P < 0.05$.

3.3 Fertility Indices

There was a slight reduction, but not significant, for number of offspring size in the EM and PFO groups compared with the CM. In the PFM group there was a slight reduction, but not significant, of fertility index and that of viable gestation. Testosterone levels were significantly decreased in EM and in the PFM ($P < 0.001$). Results are reported in Table 2.

3.4 Intestinal status

The intestinal weight, intestinal length, serosa area of duodenum and intestinal water content, were found to be significantly decreased in ethanol-exposed group compared to controls. Results are reported in Table 3.

Table 2: Fertility indices in the parents and in the offspring rats

Parameters (Mother)	CM	EM	PFM
Testosterone (Serum)	18.40±0.76	8.29±0.51	6.94±0.57
(Nmoles/l)	(n = 7)	*** $P < 0.001$ (n = 1)	*** $P < 0.001$ (n = 9)
Fertility Index (%)	100	100	100
Gestational Index (%)	96.1	100	83.34
Survival Index (%)	70.87	56.74	64.56
Offspring	15.49±0.45	14.18±0.51	14.68±0.97
	(n = 15)	(n = 20)	(n = 25)

The results are expressed as mean ± SEM. Mean differences were studied by using analyses of variance (ANOVA) followed by the Tukey's test. n' indicate the number of animals in each group. ED versus CD: *** $P < 0.001$; PFD versus CD: *** $P < 0.001$.

Table 3: Offspring intestinal status at the end of suckling period

Offspring (n=15)	CO	GO	LO	PFO
Total Intestinal Length (cm)	80.7±0.68, $P < 0.001$	78.98±0.42, *** $P < 0.001$	72.22±0.71, *** $P < 0.001$	75.17±0.18, *** $P < 0.001$
Serosa area of duodenum (cm²)	4.07±1.15, $P < 0.001$	3.99±0.10, *** $P < 0.001$	3.70±1.18, *** $P < 0.001$	3.83±1.24, *** $P < 0.001$
Total Intestinal Weight (g)	3.50±0.21, $P < 0.001$	3.20±0.18, *** $P < 0.001$	3.30±0.17, *** $P < 0.001$	3.40±0.24, *** $P < 0.001$
Water Small Intestine (%)	88.40±0.25, $P < 0.001$	85.13±0.16, *** $P < 0.001$	84.62±1.19, *** $P < 0.001$	87.90±0.01, *** $P < 0.001$

The results are expressed as mean ± SEM. Differences are analyzed by a multifactorial analysis of variance (ANOVA) followed by the Tukey's test. n' indicate the number of animals in each group. GO versus CO: *** $P < 0.001$; LO versus CO: *** $P < 0.001$; PFO versus CO: *** $P < 0.001$.

GO versus LO: ³ $P < 0.05$; PFO versus LO: ¹ $P < 0.001$. GO versus PFO: ° $P < 0.05$.

4. Discussion

Previous studies of the effect of alcohol on gonadal functions in experimental animals [13] demonstrated that

pair-feeding alcohol to male rats and isocalorically maintained controls for 41days produced significant damage. To the reproductive system. In vivo studies [14] showed that alcohol given in different ways, such as gavages, pair-fed liquid diets, and drinking water or through

intraperitoneally could decrease blood testosterone levels, in rat and humans. The present results show a significant decrease in the values of serum testosterone after ethanol and pair-fed treatments and could indicate decreased fertility. Lactation is a major component of reproduction unique in mammals. A few studies have examined the effects of alcohol administration during lactation. In our study, ethanol consumption decreased significantly milk consumption in both prenatally and postnatally ethanol-exposed offspring at 30 days postpartum. This unimpaired nursing may be partially responsible for the impairment growth observed in ethanol-exposed litters. Rats exposed to ethanol during gestation had normal birth weights. Litters of dams exposed to ethanol postnatally were growth-retarded, whereas litters of dams exposed prenatally to ethanol achieved normal growth at the end of suckling. Nevertheless, they showed a significant lower weight gain compared with the control group during the last week of suckling. These results are in accordance with other studies performed in rats [15] and in mice [16]. Our results in rats exposed prenatally to ethanol also indicated a significant lower weight gain at the end of suckling and, according to a previous report; it could show a tendency for subsequent retarded growth. Furthermore, the studies on pair-fed showed a descent in body weight. Ethanol and pair-fed provoked a maternal malnutrition, this fact modified the offspring body weights during the suckling. Several studies have shown that rats prenatally exposed to ethanol take a longer time to attach the nipple [17] and are incapable of exerting adequate suckling pressure and have a reduced number of rapid rhythmic sucks per minute of suckling [18]. Maternal alcohol intake during lactation greatly impairs milk production in rats [19]. Alcohol has an adverse effect on prolactin (PRL) and oxytocin release, two hormones essential for normal lactation. Researchers working with a chronically catheterized lactating rat model, milk intake and growth [20-21] have also reported that alcohol inhibits suckling-induced PRL release. However, recent studies have suggested that inhibition of oxytocin, rather than PRL, may be the primary avenue by which alcohol induces growth retardation during lactation. The other possible mechanism involved in alcohol's inhibition on milk secretion could be its effect on the structure and function of the mammary gland [22]. Recently, animal studies have also demonstrated that ethanol in utero induces epithelial cell damage [23-24] and altered kinetics in the developing rat intestine [25]. In this sense, ethanol may damage the intestinal cells and increase the space between the cells where para-cellular passive absorption may increase [26]. Interestingly, researchers using a similar experimental model of ethanol administration only during pregnancy have shown that postnatal maturation of the small intestine in the ethanol offspring was depressed during the early nursing period, even though ethanol had been withdrawn at birth, although it returned to control by Day 15 postpartum [27]. An immature aspect of the enterocytes that persisted until weaning was also found in this study. Our results showed no ethanol effects in serosa area of duodenum and total intestinal length at the end of suckling, whereas lower water content was observed in offspring exposed to ethanol postnatally because of an ethanol effect. Prolonged ethanol ingestion results in altered water-electrolyte homeostasis and induces body dehydration [28]. Maternal ethanol ingestion before and

during gestation and suckling affects the renal function of the offspring, up-regulating renal AQP₂ expression by an AVP-independent mechanism. The malnutrition increased the total length; however, the ethanol decreased it. In conclusion, the effects of ethanol and malnutrition on intestinal length are contradictory [29].

5. Conclusion

The findings of the current studies assessed the effects of maternal long-term ethanol consumption during pregnancy or lactation period on intestinal status, fertilization indices and milk consumption in offspring during 30 days postpartum. The intestinal weight, intestinal length, serosa area of duodenum, intestinal water content, milk consumption and serum testosterone level were found to be significantly decreased in ethanol-exposed group compared to controls. Further studies are needed for a better understanding of ethanol effects during pregnancy or lactation on postnatal development of fertility index of rats exposed to ethanol *in utero*.

Conflict of interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

Ethical Consideration

The manuscript is original, and it has not been published elsewhere. All authors reviewed the original content of the manuscript. No AI tool was used during the study and writing the manuscript.

Acknowledgment

I, Zamiruddin Ansari, am extremely grateful for the financial supports by Indian Council of Medical Research (ICMR), Ministry of Health and Family Welfare, Government of India for Senior Research Fellowship (SRF) during this study.

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