Qatar Univ. Sci. Bull. (1989), 9: 189-198

EFFECT OF DAILY ALCOHOL INJECTION OF PREGNANT RATS ON THE DEVELOPING EMBRYOS

By

HAMZA AHMAD EL-SHABAKA*
Zoology Department, Faculty of Science, University of Qatar
Doha, Qatar

Key words: Alcohol, rat embryos

ABSTRACT

In the present investigation; three doses; 0.002 (group I), 0.004 (group II) and 0.006 (group III) ml/gm body weight of 25%ethanol were used. Continuous IP injection of alcohol from day 7 through day 15 caused a significant decrease of viable embryos and mean embryonic body weight in groups I and II. In group III, no viable embryos were obtained. Injection of alcohol also caused a significant increase of resorption incidence of developing embryos as the dose increased. Continuous injection of alcohol from day 7 through day 15 of gestation caused some defects of the head region including hydrocephaly, narrow forehead, small eyes, reduced upper and lower jaws and absence of ears. Alcohol injection also caused some craniofacial anomalies including slight cleaving of upper lip, closely set nostrils and also produced extensive cellular damage of cerebral cortex.

INTRODUCTION

Maternal alcohol consumption in human results in a group of abnormalities known as fetal alcohol syndrome "FAS" (Jones and Smith, 1973). Such syndrome is characterized by pre- and post-natal growth retardation, facial dysmorphology, central nervous abnormalities and skeletal and cardiovascular anomalies (Jones and Smith, 1973, 1975; Clarren and Smith, 1978). The incidence of malformations varies according to the stage of embryonic development at the time of exposure, the route of administration, the amount of alcohol given and the time period over which it was administered (Borges and Lewis, 1982). In most cases, however, attention has been focused on the effect of one intraperitoneal (IP) injection (Bannigan and Burke, 1982), two IP injections, 4 or 6 hours apart (Sulik and Johnston, 1983; Webster *et. al.*, 1983; Nakatsuji and Johnson, 1984), one or two oral doses with four hours interval (Webster *et. al.*, 1983), a daily oral dose from gestation day 7 through gestation day 15(El-Shabaka, 1988) and in diet from gestation day 5 through gestation day 10 (Randall and Tylor, 1979). However, the specific effects of a daily IP injection of ethanol remain unknown. Hence, the aim

*Present address: Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

of the present investigation is to study the teratologic effects of a daily IP injection of low dose of ethanol for a long period (from day 7 through day 15 of gestation).

MATERIAL AND METHODS

Virgin white rats weighing 250-300 gm were kept with males (one male for three females) for two hours in the early morning. Vaginal smears were then examined carefuly for the presence of sperms which later indicated positive mating, and the date was considered as the 0 day of gestation.

The pregnant rats were divided into 6 groups (I-VI), 10-15 each. Groups I-III of pregnant rats were injected daily, from day 7 to day 15 of gestation, with 0.002, 0.004 and 0.006 ml/gm body weight of 25% (v/v) ethyl alcohol respectively. Groups IV-VI were injected daily, from day 7 through day 15 of gestation, with 0.002, 0.004 and 0.006 ml/gm body weight of normal saline solution and served as control for the corresponding groups I-III respectively.

Alcohol-injected rats were sacrificed on days 12 and 15 of gestation by cervical dislocation, and the uteri were removed and placed in a normal saline solution. Embryos were freed from the uterine tissue, and then fixed in either Zenker's or Bouin's fluids. Fixed embryos were dehydrated in ascending series of ethyl alcohol, cleared in terpeniol, embedded in paraffin wax, serially sectioned at 7 microns and stained with haematoxylin and eosin.

The significance of the differences between controls and alcohol-treated embryos was determined using student t-test.

RESULTS

The mean of viable, dead and malformed embryos as well as embryonic body weight and resorption of control and alcohol-treated embryos during days 12 and 15 of gestation are presented in Table I. A significant decrease of mean viable embryos was noted in group I at days 12 ($p<0.01$) and 15 ($p<0.01$) and in group II at days 12 ($p<0.001$) and 15 ($p<0.01$) of gestation, as compared with the corresponding controls (groups IV and V respectively). However, all embryos of group III were resorbed at days 12 and 15 of gestation (Table 1).

A significant decrease of mean embryonic body weight was observed in group I at day 12 ($p<0.05$) of gestation and in group II at days 12 ($P<0.01$) and 15 (p0.05) of gestation compared with the corresponding controls in groups IV and V respectively (Table 1). Moreover, a significant resorption incidence ($p<0.001$) was observed at gestational days 12 and 15 of pregnancy in groups I-III as compared with the corresponding controls in groups IV-VI respectively (Table 1).

On the other hand the mean number of dead embryos showed insignificant increase in group II at days 12 and 15 of gestation compared with the corresponding controls in group V (Table 1).

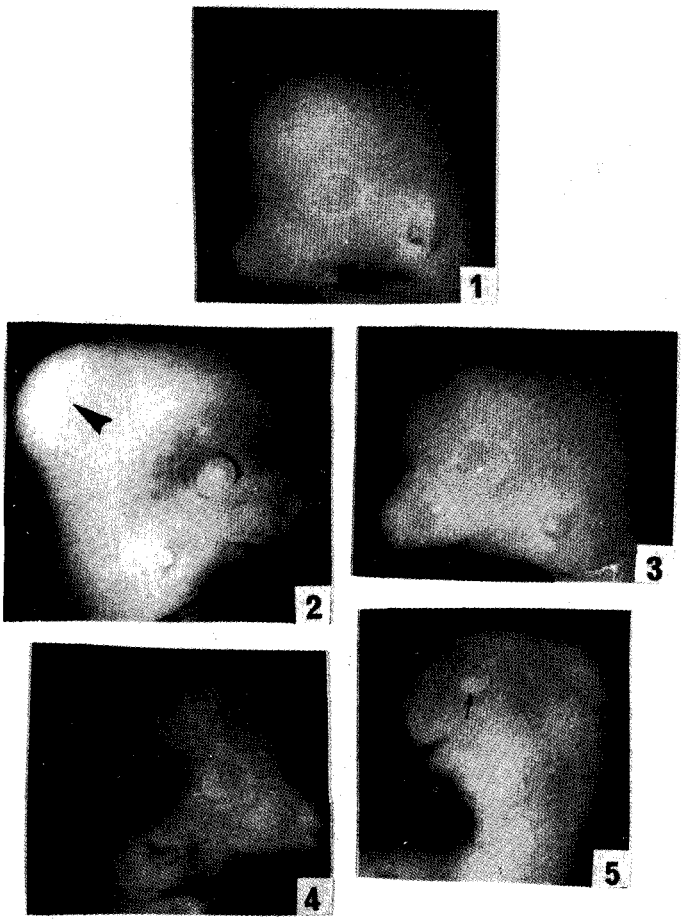
Table 1

The effect of continuous alcohol injection from day 7 through day 15 of gestation on the developing rat embryos.

Dose	Number of litters		Mean of viable embryos ± SE		Mean of dead embryos ± SE		Mean of abnorm embryos ± SE	Mean of embryonic body weight ± SE		Mean of resorptions ± SE	
	day 12	day 15	day 12	day 15	day 12	day 15	day 15	day 12	day 15	day 12	day 15
0.002 ml alcohlo/gm (group I)	8	6	8.2 ±0.296 (p< 0.01)	7.8 ±0.342 (p< 0.01)	0.0 ± 0.0	0.0 ± 0.0	4.375 ± 0.498 (p< 0.01)	0.016 ± 0.003 (p< 0.05)	0.169 ± 0.031	3.3±0.59 (p< 0.001)	3.5±0.428 (p<0.001)
0.004 ml alcohol/gm (group II)	8	7	6.6 ±0.403 (p< 0.001)	5.6 ±0.687 (p< 0.01)	0.25±0.16	0.57±0.3	5.625 ± 0.625 (p< 0.001)	0.016±0.002 (p< 0.01)	0.11±0.022 (p< 0.05)	5.0±0.707 (p<0.001)	5.714±0.565 (p< 0.001)
0.006 ml alcohol/gm (group III)	6	6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.00±0.00	0.00±0.00	6.833±0.65 (p< 0.001)	6.7±0.495 (p< 0.001)
0.002 ml saline/gm (group IV)	5	5	10.2±0.374	10.0±0.365	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.025±0.002	0.188±0.021	0.00±0.00	0.00±0.00
0.004ml saline/gm (group V)	5	5	10.0±0.316	9.8 ±0.374	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.028±0.002	0.185±0.02	0.00±0.00	0.2±0.20
0.006 ml saline/gm (group VI)	5	5	10.4±0.510	9.6 ±0.748	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.023±0.0009	0.191±0.03	0.00±0.00	0.00±0.00

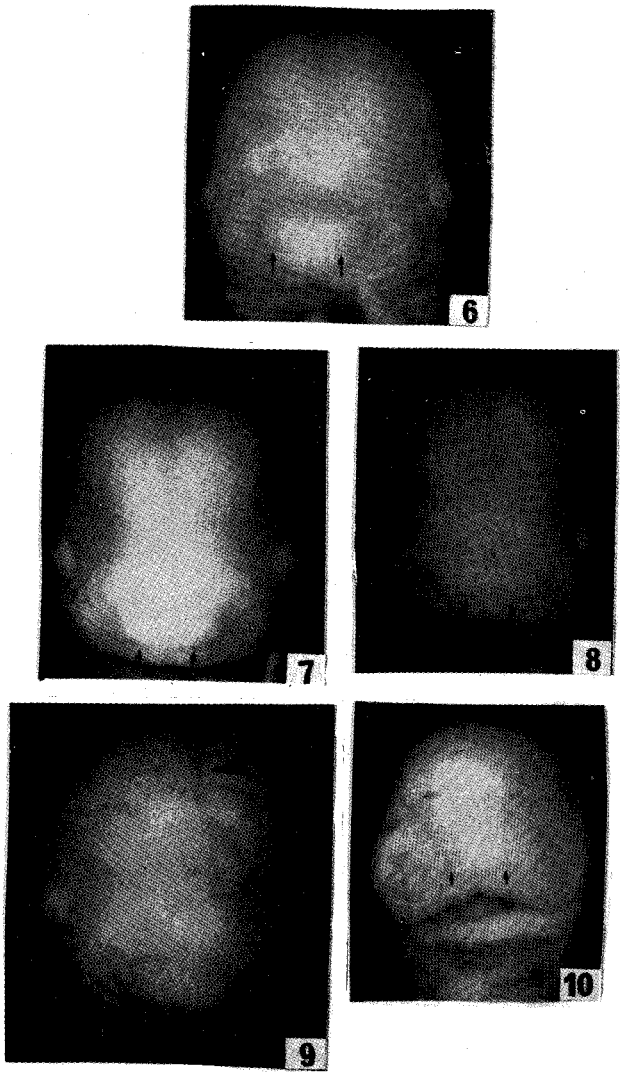
Incidence of Malformations

Daily IP injection of alcohol from day 7 of gestation produced significant defects (p 0.001) of the head region of 15-day embryos in both groups I and II (Table 1). In group I and among 8 alcohol-injected mothers, 4 had abnormal 15-day embryos including hydrocephaly (Fig. 2) and narrow forehead (Fig. 3). In group II and among 8 alcohol-injected females, 6 had abnormal 15-day embryos including hydrocephaly, haematoma (Fig. 4) and small head, small eyes, reduced upper and lower jaws and absence of ears (Fig. 5). On the other hand, all control groups had no head abnormalities.



Figs. 1-5: Rat embryos on day 15 of gestation showing the heads of control (Fig. 1), group I (Figs. 2 and 3) with hydrocephaly (arrowhead) and group II (Figs. 4 and 5) with hydrocephaly (arrowhead), hematoma (arrow) and reduced eye (small arrow). X 8

Some craniofacial malformations were noted in groups I and II at day 15 of gestation. These abnormalities include slightly clefted upper lip, closely set nostrils (Figs. 7 and 8) and narrow forehead (Fig. 8) of group I. In group II, the craniofacial malformations include narrow area between nostrils (Figs. 9 and 10), slightly clefted upper lip (Fig. 9) and abnormal upper and lower jaws (Fig. 10).



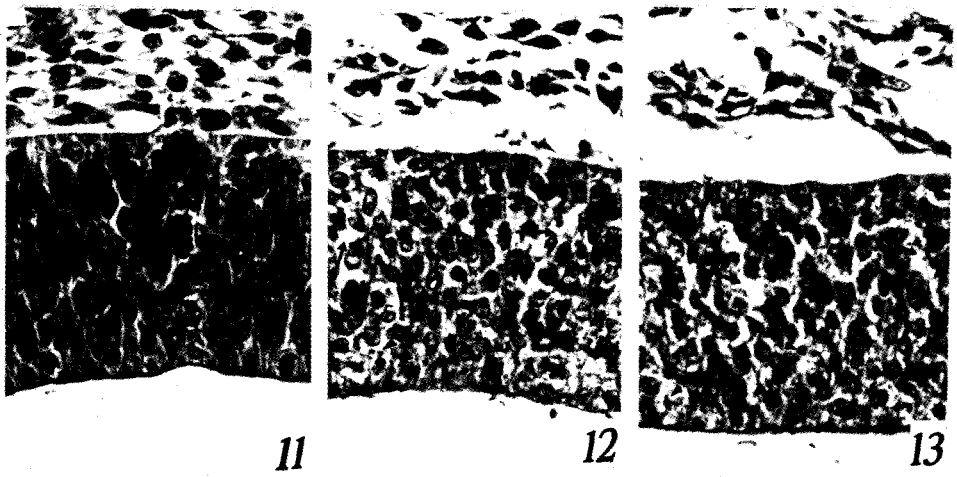
Figs. 6-10: Rat embryos on day 15 of gestation showing the heads of control (Fig. 6), group I (Figs. 7 and 8) showing closely set nostrils and group II (Figs. 9 and 10) showing hydrocephaly (arrowhead) and closely set nostrils. X 12

Histology

12-day embryos

The cerebral cortex of control embryos is composed of a stratified epithelium, the cells of which contain oval nuclei. The cells of the inner or ependymal layer contain nuclei with highly mitotic figures and their apical borders possess numerous pseudopodial processes (Fig. 11).

The injurious effects of alcohol were noted only in the brain of the developing embryos. In groups I and II, the neuroepithelium consists of a stratified epithelium with irregular or round nuclei (Figs. 12 and 13); numerous stages of degenerating nuclei and many nuclear debris were seen scattered elsewhere, especially in the cerebral cortex of group II embryos (Fig. 13). In addition, the intercellular spaces of the neuroepithelium were enlarged and the mitotic figures in the ependymal cell layer were reduced than in the control embryos.

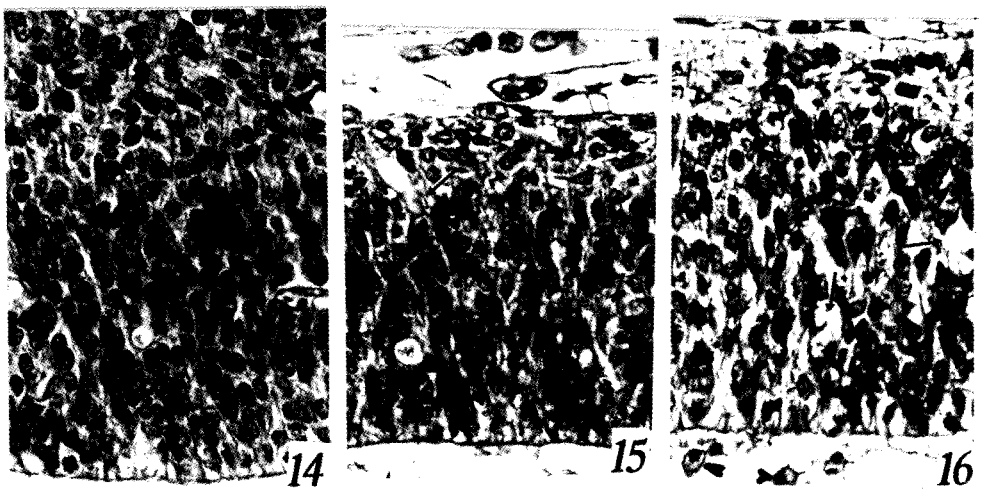


Figs. 11-13: T.S. through the forebrain of developing rat embryos on gestation day 12 of control (Fig.11), group I (Fig. 12) and group II (Fig. 13) showing necrotic particles (arrowheads) and cytoplasmic vacuoles (arrows). X 490

15-day embryos

After 15 days of pregnancy, the cerebral cortex of control embryos is thickened due to the active proliferation of the ependymal layer (Fig. 14).

In group I (Fig. 15), the cells of the cerebral cortex contain few necrotic particles, cytoplasmic vacuoles and mitotic figures in the ependymal cell layer as compared with the control embryos. In group II, the cerebral cortex shows some necrotic particles, intracellular vacuoles and a few blood cells dispersed inside the lateral ventricles of the forebrain (Fig. 16).



Figs. 14-16: T.S. through the forebrain of developing rat embryos on gestation day 15 of control (Fig. 14), group I (Fig. 15) and group II (Fig. 16) showing numerous vacuoles (arrows) and red blood corpuscles inside ventricle (arrowheads). X 680

DISCUSSION

In the present investigation, three doses; 0.002, 0.004 and 0.006 ml/gm body weight of 25% ethanol were applied. Injection of alcohol on day 7 of gestation caused a significant decrease of mean number of viable embryos of groups I and II during 12 and 15 of gestation as compared with the corresponding controls. However, no viable embryos were obtained from group III on the 12 *th* and 15*th* days of gestation.

The mean embryonic body weight of groups I and II on day 12 of gestation and group II on day 15 of gestation were significantly reduced as compared with the corresponding controls. This decrease in the embryonic body weight contradicts with the results reported by many authors (Kronick, 1976; Randall and Taylor, 1979; Bannigan and Burke, 1982; El-Shabaka, 1988), probably due to the difference in the route of alcohol administration and / or to the successive daily injection. Moreover, Webster *et. al.* (1983). reprted that the abnormal and some normal fetuses of alcohol-treated groups were significantly lighter than control fetuses of corresponding groups.

Injection of alcohol also caused a significant increase of resorption incidence of developing embryos as the dose increased, until an embryotoxic dose (0.006 ml/gm body weight) was reached when total resorption occurred. An increase of resorption incidence has been reported in different treatment periods, doses and routes of ethanol administration (Randall and Taylor, 1979; Bannigan and Burke, 1982; Webster *et. al.*, 1983; El-Shabaka, 1988).

Continuous IP injection of alcohol from day 7 through day 15 of gestation caused some defects of the head region of rat embryos including hydrocephaly, narrow forehead, small head, small eyes, reduced upper and lower jaws and absence of ears. In general, the patterns of head anomalies were increased as the dose increased. These results confirm some findings in mice and rat embryos by employing different treatments and different routes of alcohol administration (Randall and Taylor, 1979; Bannigan and Burke, 1982). On the other hand, some defects due to alcohol administration including exencephaly (Randall and Taylor, 1979; Bannigan and Burke, 1982; El-Shabaka, 1988), hydro-nephrosis (Randall and Taylor, 1979) have not been reported in the present investigation.

Alcohol injection also caused some craniofacial anomalies including slight cleaving of the upper lip, closely set nostrils and abnormal upper and lower jaws. These anomalies confirm those reported by Sulik and Johnston (1983) and Webster *et. al.* (1983). The increased frequency of craniofacial anomalies in the present investigation as compared with those reported by others (Randall and Taylor, 1979; Scott and Fradkin, 1984; Clarren *et. al.*, 1987; El-Shabaka, 1988) may be due to the difference in route of alcohol administration.

Injection of alcohol, from day 7 through day 15 of gestation, produced extensive cellular damage of cerebral cortex with no alteration of the neuroepithelial thickness. On the other hand, Bannigan and Burke (1982) reported retardation of the neuroepithelium of alcohol-treated embryos. Ethanol-treatment caused susceptible effect for cell division of ependymal layer. Moreover, numerous cytoplasmic vacuoles and necrotic particles were visible in the neuroepithelial cells of alcohol-treated embryos. These results agree with those reported by Bannigan and Burke (1982) and El-Shabaka (1988). On day 15 of gestation, the neuroepithelium of groups I and II showed no signs of repairing since numerous vacuoles and necrotic particles were still visible. This result, which contradicts that reported by Bannigan and Burke (1982) and El-Shabaka (1988) may be due to prolonged exposure period and/or variation of alcohol administration route.

ACKNOWLEDGEMENT

The author would like to thank prof. Dr. F.I.Amer, Faculty of Science, Ain Shams University for his helpful criticism and Prof. Dr. W.Rizkalla, Faculty of Science, Ain Shams University for his continued support and encouragement.

REFERENCES

Bannigan, J. and **Burke, p. 1982.** Ethanol teratogenicity in mice: A light microscopic study. Teratology, 26: 247-254.

Borges, S. and **Lewis, P.D. 1982.** A study of alcohol effects on the brain during gestation and lactation. Teratology, 25: 283-289.

Clarren, S.K., Bowden, D. and **Astley, S.J. 1987.** Pregnancy outcomes after weekly oral administration of ethanol during gestation in the pig-tailed-macaque *Macaca nemestrina* Teratology, 35: 345-354.

Clarren, S.K. and **Smith, D.W. 1978.** The fetal alcohol syndrome. New Eng. J. Med. 298: 1063-1067.

El-Shabaka, H.A. 1988. Effect of oral administration of alcohol on the developing rat embryos. Qatar Univ. Sci. Bull. vol, 8: 231-246

Jones, K.L. and **Smith, D.W. 1973.** Recognition of the fetal alcohol syndrome in early infancy. Lancet, 2: 999-1001.

Jones, K.L. and **Smith D.W. 1975.** The fetal alcohol syndrome. Teratology, 12: 1-10.

Kronick, J.B. 1976. Teratogenic effects of ethyl alcohol administered to pregnant mice. Amer. J. Obster. Gynec., 124: 676-680.

Nakatsuji, N. and **Johnson, K.E. 1984.** Effects of ethanol on the primitive streak stage mouse embryo. Teratology, 29: 369-375.

Randall, C.L. and **Taylor, W.J. 1979.** Prenatal ethanol exposure in mice. Teratogenic effects. Teratology, 19: 305-312.

Scott, W.J. and **Fradkin, R. 1984.** The effects of prenatal ethanol in cynomolgus monkeys. Teratology, 29: 49-56.

Sulik, K.K. and **Johnston, M.C. 1983.** Sequence of developmental alterations following acute ethanol exposure in mice: Craniofacial features of the fetal alcohol syndrome. Am. J. Anat., 166: 257-269.

Webster, W.S., Walsh, D.A., Mc Ewen, S.E. and **Lipson, A.H. 1983.** Some teratogenic properties of ethanol and acetaldehyde in C57 BL/6J mice: Implications for the study of the fetal. alcohol syndrome. Teratology, 27: 231-243.

تأثير الحقن يومياً بالكحولات على نمو أجنة الفئران

حمزه أحمد السيد الشبكة

أُستخدم في هذا البحث ثلاث جرعات من الكحول الإثيلي تركيزه ٢٥٪ حقنت يومياً بداية من اليوم السابع وحتى اليوم الخامس عشر من الحمل . وقد وجد أن حقن جرعات مقدارها ٠٠٢, مل/جرام تسبب نقصاً في عدد ووزن الأجنة كما أدت هذه الجرعات إلى زيادة ملحوظة في عدد الأجنة الممتصة داخل الرحم . أما الجرعة ٠٠٦, مل/جرام فقد أدت إلى إمتصاص كل الأجنة ولم يُتمكن من الحصول على أجنة أحياء في كل المحاولات التي تمت . وقد أحدثت الجرعتان الأولى والثانية بعض التشوهات للأجنة مثل إنتفاخ الرأس وصغر الأعين وإختزال الفكوك وغياب الأذن بالاضافة إلى تقارب فتحات الأنف الخارجية . وقد أحدثت هذه الجرعات أيضاً تأثيراً واضحاً على مخ الأجنة فقد حدث تحطيم لكثير من خلايا القشرة المخية مما أدى إلى تكوين فجوات كثيرة بينها .