

EFFECT OF ALCOHOL CONSUMPTION IN PREGNANCY ON PUP QUALITY, EXPLORATORY BEHAVIOUR, MEMORY RETENTION IN WISTAR RATSMitha K.V¹, Saraswati Yadav¹, Santhosh Mayannavar² and Ganaraja B³¹Junior Research Fellow, Department of Physiology, Kasturba Medical College, Mangalore. (A Unit of Manipal University) Karnataka, India. Email: mithakv@yahoo.com, saras.jaiswal@gmail.com.²Senior Research Fellow, Department of Physiology, Kasturba Medical College, Mangalore. (A Unit of Manipal University) Karnataka, India. email: mayannavar.santosh@gmail.com.³Additional Professor, Department of Physiology, Kasturba Medical College, Mangalore. (A Unit of Manipal University) Karnataka, India.(Corresponding Author) Ph: +919449642150 Email: ganaraja.b@manipal.edu & ganaraj.b@gmail.com

ABSTRACT: Prenatal exposure to alcohol can affect both prenatal and postnatal neurogenesis in the developing brain and impair brain function in their early life. **Aim:** Our study was aimed to assess the effect of prenatal alcoholic exposure on memory retention and exploratory behavior in young adult rats. We also studied the effect of maternal alcohol intake on pup quality, mortality rate and post natal weight gain of the pups during their weaning period. **Methods:** Female rats were divided into control and alcohol fed group. Rats in alcoholic group were orally fed (force feeding) with 30% alcohol at a dose of 5g/kg /day. Treatment was started 14 days before mating, continued throughout their gestation period and weaning period. Control group was administered with equivalent volume of water. Offspring from each group were divided into male and female group. Birth weight, crown-rump length, litter size were taken from the day of delivery, whereas cognitive function test were done from 75th day of post natal life. **Statistics:** Data obtained from the tests were analyzed by applying independent T test. **Results:** Single dose of 5g/kg/day maternal ethanol treatment decreased the memory retention ($p=0.003$), decreased the weight gain during weaning period ($p=0.000$), increased the locomotor activities ($p=0.05$) and increased the mortality rate of pups during weaning period. No significant change was observed in the pup quality between control mother and alcoholic mother. **Conclusion:** The present study showed that maternal alcohol consumption could affect mortality rate of the pups and their post natal weight gain during weaning period. It also affects their cognitive behaviour and locomotor activities in their later life.

Key Words: Alcohol, memory retention, open field, pup quality, passive avoidance

INTRODUCTION

Ethanol is considered to be one of most common teratogenic substance, and prenatal alcohol exposure is a leading preventable cause of birth defects, mental retardation and neurodevelopmental disorders (American Academy of Pediatrics. 2000). It is mainly associated with microcephaly, hypomyelination, delayed cell migration, decreased maturation of neurons and glial cells. Fetal Alcohol Syndrome (FAS)(Jones KL et al.1973) is one of the consequences of the prenatal alcohol exposure, characterized by pre and post natal growth deficiencies, cranio facial anomalies and evidence of central nervous system dysfunction. Heavy prenatal alcohol exposure has been associated with wide spread neuropsychological deficits across several domains, including general intelligence, memory, language, attention, learning, visuospatial abilities, executive functioning, fine and gross motor skills, and social and adaptive functioning (Mattson SN et al .1998). The severity and pattern of defects are mainly dependent upon the amount and pattern of alcohol consumption during pregnancy and as well as the specific time during gestational period in which the fetus is most heavily exposed (David E et al.1980). During the first trimester of pregnancy, alcohol interferes with the migration and -organization of brain cells, which can create structural deformities or deficits within the brain (Clarren S et al 1992).

During the third trimester, damage can be caused to the hippocampus, which plays a role in memory, learning, emotion, and encoding visual and auditory information (Coles C. et al. 1991). Previous studies showed that neurobehavioral abnormalities are mainly observed in early life of animals but appear to diminish as the animal matures (Bond NW. 1981). In this particular study we mainly focused on the exploratory behavior and memory retention in young adult rats that were prenatally exposed to alcohol and also observed the post natal weight gain, and also the mortality rate of the offspring during their weaning period.

MATERIALS AND METHODS

Albino rats of Wistar strain of either sex were selected. The rats were maintained in 12 hours light and dark cycle in temperature and humidity controlled environment, and were fed with standard food pellet (Amruth laboratory, Maharashtra, India) and tap water ad libitum. Breeding and maintenance of the animals were done as per the guidelines of Government of India for use of Laboratory animals. Institutional Animal Ethical Committee (IAEC) approval had been obtained. Virgin female rats with initial body weight of 150-200 gram were taken and they were divided into 2 groups (n=6 each) control group (C) and alcohol group (A). Female rats in 'alcohol group' were fed with 30% v/v ethanol (Hayman Ltd, England) at a dose of 5g/kg/day (Nio et al, 1991, Ighodaro Osasenaga et al, 2010). Forced oral feeding was started 14 days before mating, continued throughout their gestation and lactation period. Control group was administered with equivalent volume of water for same period of time. Offspring from each group (control and alcoholic mothers) were divided into Offsprings from Control mothers (OCM, n=12, 6 male +6 female); Offsprings from Alcohol fed mothers (OAM n=12, 6 male +6 female).

On the day of delivery, Numbers of live pups, birth weight, crown-rump length, and litter size were noted and recorded. To study the weight gain during the weaning period, all the pups were weighed on 1st, 7th, 14th, and 21st day. All the pups were kept with their mother until 21 days of postnatal age. Then, they were grouped into males and females and kept in separate cages. Cognitive function tests were done from 75th day of post natal life.

Behavioral test

Offspring in the entire four groups (viz. Control & Alcohol groups – males and females in each) were subjected to following behavioral tests:

- Passive avoidance test
- Open field behavioral test

Passive avoidance test:

To test the memory retention, rats were subjected to passive avoidance test (Madhyastha S et al, 2007). Passive avoidance apparatus consists of a wooden box with a larger, bright compartment and a smaller, dark compartment with grid floor, which is attached to a shock source. On the first day of test rat was allowed to explore both chambers for five minutes. This was followed by three test trials of five minute each. In 4th trial, as soon as rat stepped into dark compartment, a foot shock (2.5mA) was given and rat was replaced to home cage. After 24 hours, rats were placed in the test chamber and latency to enter the dark compartment was measured. Normal rats avoid entering the dark chamber, where they received shock on previous day, suppressing their normal behavior of exploring the dark compartment. Decreased latency to enter the dark compartment will suggest poor memory retention (Saju Binu Cherian et al, 2009).

Open field behavior

Open field test is one of the most widely used methods to assess the motor and exploratory activities and emotional reactivity of rodents (Tobach E, 1996). Open field behavioral test apparatus is a rectangular box with a floor consisting of 25 equal squares. Illumination is provided by 100 watt bulb fixed at 60 cm above the centre of the field (Bures et al, 1983). The rat was placed in a corner of the apparatus and was allowed to explore the apparatus for 5 minutes. During this period the number of peripheral and central squares entered by the rat and the number of expressions of grooming, rearing and boli of excreta excreted were counted. Increase in the number of peripheral squares entered and more time spent in the peripheral area was considered as an increase in motor activity. Increased number of central squares entered and more time spent in the centre of the field indicates decreased fear and anxiety and emotional disturbance. Decreased rearing and Increased number of grooming (Buresova O. et al, 1976), increased number of boli of excreta (Sutton RE et al, 1982) are the expressions of emotionality, which are measures of autonomic function in the animals.

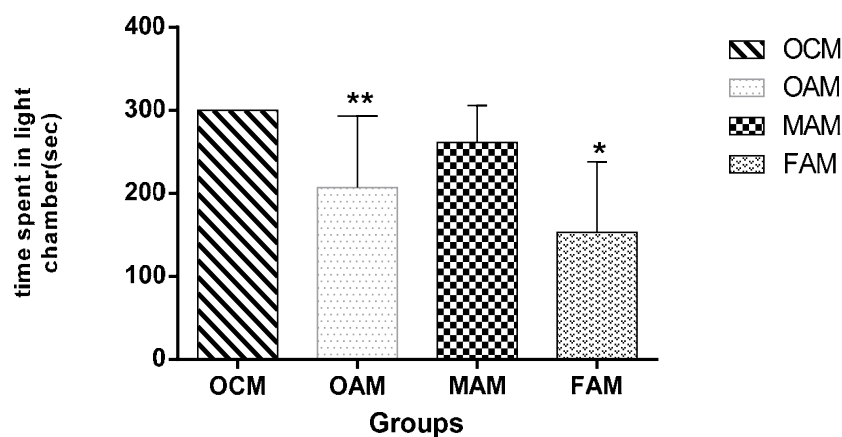
STATISTICS

Statistical analysis was done by using IBM SPSS (version 16) and the data shown as the means \pm SD and $p \leq 0.05$ was taken as statistically significant value. Data obtained from the above tests were analyzed by applying independent T test.

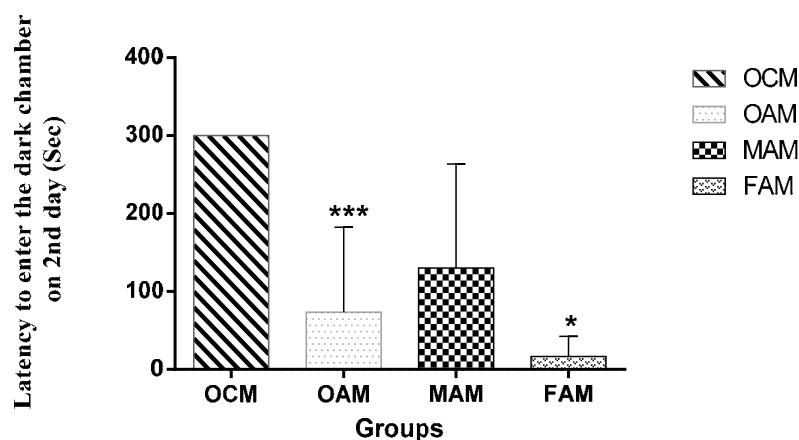
RESULTS

Passive avoidance test:

Offspring from alcohol treated mothers spent significantly ($p < 0.01$) less time in light chamber on 2nd day of passive avoidance as compared to offspring from control mothers. There was a significant difference ($p < 0.05$) between female offspring and male offspring (graph 1). Latency to enter into the dark chamber was significantly ($p < 0.001$) less in offspring from alcoholic mothers as compared to offspring of control mothers. Female offspring from alcoholic mothers showed significantly ($p < 0.05$) less latency as compared to the male offspring (graph 2).



Graph 1. Time spent in the light chamber



Graph 2. Latency to enter the dark chamber on the second day

Graph 1. Time spent in the light chamber, Graph 2. Latency to enter the dark chamber on the second day, unpaired t test done, OCM=Offspring of control mother, OAM= Offspring of alcohol mother, MAM=Male offspring of alcoholic mother, FAM= female offspring of alcoholic mother, * $p < 0.05$, ** $p < 0.01$

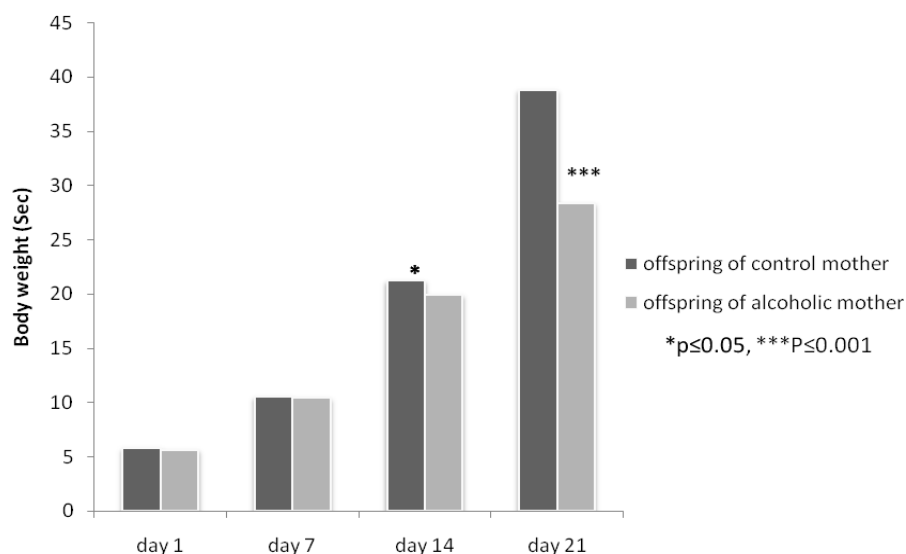
Open field test: Offspring from alcohol treated mothers had made significantly ($p < 0.05$) more peripheral square entry as compared to the offspring from control mothers. And this was significantly ($p < 0.05$) more in females offspring as compared to male offspring from alcohol treated mothers. There was no statistically significant difference in any other parameter studied in the open field behavioral test (Table-1).

Table 1: Open field test results

GROUP (n=6)	Peripheral Squares	Central Squares	Grooming score	Rearing score	Defecation score
OCM	35.08±10.6	4±0.96	5.33±1.28	6.41±1.01	3.1±0.63
OAM	43.91±10.6*	6.8±1.9	4.66±0.82	6.66±1.36	4.6±.568
MAM	36.8±1.95	2.8±1.9	4.1±0.94	4.1±2.3	2.3±1.7
FAM	51±4.1*	4.08±1.6	4.8±0.70	4.8±1.7	3.3±1.6

Independent t test done, * $p < 0.05$, data as Mean \pm SE, OCM= offspring from control mothers, OAM=offspring from alcoholic mothers, MAM =male from alcoholic mother, FAM=female from alcoholic mother

Post natal Body weight gain: Post natal weight gain during weaning period was less in offspring from alcoholic mother as compared to the offspring from control mothers. There was a no significant change in the body weight on 1st and 7th day of post natal life. A significant change ($p \leq 0.001$) was observed on 14th and 21st day of post natal life, offspring from alcoholic mother had decreased weight gain during weaning period (graph-3).



Graph-3: Weight gain during weaning period

Mortality rate: Mortality rate of pups during weaning period is more (10%) in alcoholic group as compared to the pups from control mothers (2.43%) (Table-2).

Table 2: Mortality rate

Groups (n=6)	Number of pups born	Number of dead pups	Dead pups/total born (%)
Control mothers	41	1	2.43%
Alcohol fed mothers	50	5	10%

Birth weight, Crown length, and Litter size: There was no statistically significant difference in the birth weight ($p = 0.637$) and crown rump length ($p = 0.567$) of pups from control and alcoholic mother. The number of live pups on the day of delivery is taken as the litter size. There was no significant difference in the number of live pups ($p = 0.333$) from alcohol treated and control group rats (Table-3).

Table 3: Results of birth weight, crown-rump length and litter size

GROUP (n=6)	Birth weight mean	Crown-rump length mean	Litter size mean
OCM	5.6814±1.7	4.69±0.62	7.5±1.8
OAM	5.5490±0.60	4.75±0.36	8.5±1.5

Independent t test done, data as Mean ± SE, OCM= offspring from control mothers, OAM=offspring from alcoholic mothers.

DISCUSSION

The present study has demonstrated that maternal alcohol consumption can cause neurobehavioral problems in the offspring in their later life. We also observed that, maternal alcohol consumption during lactation period (which is equals to the 3rd trimester of the human gestation period) affects the mortality rate and post natal weight gain during weaning period. Memory retention test was done on 75th day of post natal life by using the passive avoidance test in which foot shock was given on the first day in the dark chamber and the latency to enter the dark chamber on 2nd day was observed. Offspring from alcoholic mother had decreased latency as compared to the offspring from control mothers which means offspring from alcoholic mother spent less time in the light chamber. This indicates decreased memory retention in them. This decreased memory retention was more in female offspring as compared to the male offspring from alcoholic mothers (Graph 1&2). Earlier studies have suggested passive avoidance was affected in rats only during weaning period but not in older animals (Riley EP et al,1979, Abel PhD 1982) but in our study it appears to last longer. Open field behavioral test for the locomotor activity, exploratory behavior and emotional status, was done in all the groups. In open field behavioral test, those animals which ambulate less and defecate more are considered more emotional than the animals with high ambulation and low defecation scores. We observed increased locomotor activity in offspring from alcohol treated rats, they crossed more peripheral squares than offspring from control. Previous studies have reported that prenatal exposure to alcohol in the 2nd trimester increases the locomotor activities in 30 days old rats (Gary E et al, 1989). In our present study we started alcohol treatment 14 days before mating and continued throughout their gestation period and lactation period, open field test was done on 77th day of postnatal life .we found increased locomotor activities in this young adult rats, and it was more significant in female offspring as compared to the male (table 1).

We observed reduced post natal weight gain by the end of weaning period in pups from alcoholic mothers than the pups from control mothers (graph 3). Ethanol is known to be inhibit lactation (Cobo E 1973) and thus post natal ethanol consumption by the mother may lead to under nutrition of the developing pups. This result is in compliance with the previous study where the mice were fed with liquid diet of 25% alcohol during their gestation period, and had reduced postnatal weight gain (Lawrence D et al, 1991). Table 2 showed that the mortality rate of pups during weaning period was more in alcoholic mothers group, indicating lesser survival rate of pups from alcoholic mother as compared to the survival rate of pups from the control mothers. However we noted that there was no statistically significant change in the birth weight, crown length and the litter size from both the control and alcohol treated mothers, From the results it appears that feeding single dose of alcohol at the mentioned dosage did not produce organ deformities in the pups of this group. Previous studies reported that a treatment with 20% v/v ethanol solution given orally; a week before mating till the weaning period had an effect on birth weight, body length and litter size of their offspring (Ghimire S.R et al 2008). However, they gave the alcohol to the rats continuously. In our study with single dose of ethanol (30% at a dose of 5g/kg body weight /day), we could not find any significant difference in birth weight, crown rump length and litter size of offspring between control mothers and alcoholic mothers. (Table-3)

CONCLUSION

The present study suggests that maternal alcohol consumption increases the mortality rate and decreases the weight gain of the pups during weaning period. But morphological changes were not observed in this study probably because we fed the alcohol as a single dose in the morning. Further study by feeding the rats in multiple dosages to maintain a steady plasma level of alcohol might produce different results. In the present study it is proved that exposure to alcohol affects the memory retention and locomotor activities of the offspring in their later life. Decreased memory retention could explain the dysfunctions of the hippocampus due to maternal alcohol consumption.

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