

# OCULAR DEFECTS IN FIRST GENERATION OF ETHANOL EXPOSED ALBINO RATS AND ITS PENETRANCE INTO THIRD GENERATION

Muhammad Ismail, Muhammad Zahoor Janjua

Department of Anatomy Saidu Medical College, Swat and BMSI, JPMC, Karachi

**Background:** The objective of this study was to observe the ocular teratogenic effects of alcohol on the third generation in albino rats. **Methods:** This study was carried out at department of Anatomy, Basic Medical Sciences Institute, JPMC Karachi from 1996-98. 36 pregnant female rats were divided into a treated and a control group (18 in each). The gestation period of albino rats is between 21-23 days (7 days trimester). Treated group was injected 25% alcohol (ethanol) intraperitoneally in a dose of 0.03 ml/gm body weight, while the control group was treated with normal saline in the same dose by same route. This intervention was done on 8<sup>th</sup>, 9<sup>th</sup> and 10<sup>th</sup> gestational day. The first generation (n=115) was crossbred to get second generation (n=104) that was then used to produce the third generation (n=95). Generations of control rats were developed parallel. No treatment was given to the subsequent generations of treated or control groups. **Results:** Ocular defects (micro/macrophthalmia, cataract, corneal opacity etc.) were observed in 30% (39/115) of the first generation, 40.38% (42/104) of the second generation and 45.26% (43/95) of 3<sup>rd</sup> generation of ethanol treated group. No ocular defect was observed in the three generations of controls. **Conclusion:** Alcohol exposure caused ocular defects in three generations of exposed rats. There is a need to study subsequent generations of rats for further penetrance and to develop cohort study in humans.

**KEY WORDS:** Alcohol, Ocular defects, pregnancy, genetic defects.

## INTRODUCTION

The most used and abused chemical is alcohol.<sup>1</sup> “Behold thou shalt conceive and bear a son and now drink no wine or strong drink”, this injunction is written in Bible (Judges 13.7). According to Aristotle (382-322 B.C), foolish, drunken and harebrain women most often bring forth children like unto themselves”.<sup>2</sup>

Alcohol (Ethanol) abuse is harmful to all tissues and systems of the body. It does not spare any system of human body from cellular organelle like mitochondria to vital organs such as brain<sup>3-7</sup> and heart<sup>8</sup>. Special multipotentials inducer cells like neural crest cell are also affected.<sup>9</sup>

Ethanol abuse during pregnancy can have adverse effects on the developing fetus and in severe cases entails in Fetal Alcohol Syndrome (FAS) producing myriads of signs and symptoms such as microphthalmia, macrophthalmia, congenital cataract, corneal opacities, limbs defects, nervous tissue and facial abnormality. FAS has been considered to be one of the main cause of mental retardation in the Western World. During pregnancy alcohol easily reaches the developing conceptus due to its low molecular weight (600-1000).<sup>10</sup> Alcoholic abuse during pregnancy also affects development of limbs and produces cleft palate.<sup>12-13</sup>

Though the exact mechanism of alcoholic damage to developing offspring is yet to be finalized, several hypothesis, like Zinc deficiency,<sup>4</sup> free radical formation<sup>9</sup>, hypoglycemia<sup>4</sup> etc. have been postulated. All these ultimately result in embryonal/foetal damage that involves perturbation in cellular growth, differentiation, proliferation, migration and regulation.<sup>13</sup>

PAX6 is the master gene for eye development, preceded by PAX 2 which induces eye development and later on the process is maintained by bone morphogenic protein 7 (BMP7).<sup>14</sup> Literature search reveals that knowledge about ocular defects in generations of alcohol treated rats is restricted to first generation only and not much is

known about the defects in subsequent generations.<sup>15-20</sup> This study was carried out to observe the ocular defects in upto 3<sup>rd</sup> generation of alcohol treated albino rats.

## MATERIAL AND METHODS

This study was carried out at department of Anatomy, Basic Medical Sciences Institutes, JPMC Karachi from 1996-98.

Treated group was injected 25% alcohol (ethanol) intraperitoneally in a dose of 0.03 ml/gm body weight, while the control group was treated with normal saline in the same dose by same route. This intervention was done on 8-10 gestational day. The first generation (n=115) was crossbred to get second generation (n=104) that was then used to produce the third generation (n=95). Generations of control rats were developed parallel. No treatment was given to the subsequent generations of treated or control groups.

The study was completed in three phases.

In the first phase we used 12 males and 36 females of proved fertility albino rats of Sprague Dawley strain. The weight of rats ranged from 173.36 to 180 grams. After one week acclimatization to laboratory condition male and females were allowed to mate overnight in ratio of 1:3 respectively.<sup>11</sup>

A sperm positive vaginal swab from mated female on next morning was considered to be day ZERO of gestation (GDO). Pregnant rats (weighing, 173.36 to 178.36 gm) were divided into two groups, Group A Alcohol treated one and Group B as a control. Group A were injected with 25% ethyl alcohol (BDH Chemical Company England) in a dose of 3ml/100 gm body weight on days 8, 9 and 10 of pregnancy (GD 8,9,10) intraperitoneally, following the protocol of Padmanabhan & Pallot.<sup>12</sup>

Group B (control) was treated with normal saline in same dose via same route on the same days.

To have a check on smoothly progressing pregnancy mothers were weighted on day 13 of gestation and pups on 1<sup>st</sup> day post delivery and checked for anomalies, if any. The ocular defects (micro/macrophthalmia, cataract, corneal opacity etc.) were noted after opening of eyes (10<sup>th</sup>-12<sup>th</sup> days). This was considered as first Filial generation (F1) of Mandel's.

In the 2<sup>nd</sup> phase mature F1 male and female rats were allowed to mate to get the second Filial generation (F2). The ocular defects were observed in the similar fashion in this generation.

In the 3<sup>rd</sup> phase mature F2 male and female rats were allowed to mate to get the third and final Filial generation (F3). Ocular defects were noted.

Three generations of the controls were developed parallel to the treated group and ocular defects were observed.

Frequencies of ocular defects in all generations of treated and control groups were compared to determine significance of difference using chi-square test at confidence interval of 95%.

## RESULTS

Three (3) intraperitoneal injections of alcohol on 8<sup>th</sup>,9<sup>th</sup> and 10<sup>th</sup> day of gestation induced general teratogenic changes in ethanol treated group, when compared with saline treated group, only ocular defects are being reported over here.

The total number of alive pups in each generation of treated and control groups is given in table-1. Table 2 shows the frequencies of ocular defects in all the generations of treated and control rats.

Frequencies of ocular defects in all generations of treated groups were significantly ( $p < 0.05$ ) more than the respective control generations. The frequency of ocular defect in F2 was significantly ( $p < 0.05$ ) more than that of F1. Similarly frequency of ocular defects in F3 was significantly ( $p < 0.05$ ) more than F2.

**Table-1: Number of alive pups in each generation**

Generation	Treated	Control
F1	115	162
F2	104	180
F3	95	198

**Table-2: Frequency of ocular defects in the generations of treated and control rats**

Generation	Treated	Controls
F1	39/115 (30%)	0/162 (0%)
F2	42/104 (40.38%)	0/180 (0%)
F3	43/95 (45.26%)	0/198 (0%)

## DISCUSSION

Penetrance refers to the appearance in the phenotype of trait/traits determined by genotype. Many developmental traits not only fully penetrate sometimes but also show variable pattern of expression from very mild to very extreme, for instance cleft palate.<sup>15</sup> Our results tally with this statement as in our experimental group some members were completely spared phenotypically i.e non expressed while other have various expression, in the form of unilateral/bilateral micro or macrophthalmia, cataract or corneal opacity.

Alcohol abuse is injurious to health. Though some categorize it between heavy and light drink, thus allowing pregnant women to drink. However others say that even 1-3 doses can produce permanent damage to brain tissue in the offspring.<sup>5</sup> No alcoholic drinks during pregnancy are recommended as there is no safe amount known to be used in pregnancy. As in our study no litter was spared so our results in the first generation are coincident with this statement.

According to Stormland<sup>2</sup> various abnormalities of anterior segment of eye, like corneal opacities, Iris defect etc were most common finding in their studies which were also found in our study.

Jones et al<sup>19</sup> showed a high proportion of offsprings of known chronic alcohol abusers to have ocular defects ranging from errors of refraction to visible morphological defects.

Our results are in accordance with that of Slotes et al<sup>21</sup>, according to whom all types of anomalies like ocular, limbs etc also were noted in his animals' study. They observed a mark ocular change in their experimental group, more in females than their male siblings. This is also supported by the study of Smith<sup>13</sup> and Clayton.<sup>14</sup>

## CONCLUSION

Alcohol exposure causes ocular defects in three generations of exposed rats. There is a need to study subsequent generations of rats for further penetrance and to develop cohort study in humans. It is recommended that use of alcohol in pregnancy must be banned and mass awareness program for this be started.

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**Address For Correspondence:**

**Dr. Muhammad Ismail**, Department of Anatomy, Saidu Medical College, Saidu Sharif, Pakistan.