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ESTIMATION OF CELL COUNT IN PARS DISTALIS OF PITUITARY GLAND AFTER PRENATAL ETHANOL EXPOSURE IN THE RAT.

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ABSTRACT

OBJECTIVE: To investigate the effects of prenatal exposure of ethanol on the total number of cells in pars disatlais of pituitary gland of rat pups raised to adults.

STUDY DESIGN: Experimental study.**PLACE AND DURATION OF STUDY:** Department of anatomy, College of Physician and Surgeons, Regional Centre, Islamabad, between April 2014 and April 2015. **METHODOLOGY:** This experimental study was done on 16 female Sprague Dawley rats, which were selected from NIH using random sampling method. Female rats were mated with four male rats and their pregnancy was confirmed by presence of spermatozoa in their vagina. The female rats were then split into two groups, experimental group 'B' and control group 'A', and used for reproduction. Group A received injections of normal saline from the 10th to the 18th gestational day GD while Group B received an intraperitoneal ethanol injections at a dose of 4.44ml/kg. Until their normal gestation and delivery at GD 21 to 23, animals were maintained in conventional laboratory settings. Pups that were males were raised until their 70th day. Periodic acid Schiff Orange G PAS-OG was used to stain pituitary glands, which were then used for histological analysis. **RESULTS:** As compared to the control group's mean acidophil count 46.93± 0.71, $p < 0.000$, the experimental group mean acidophil count revealed a reduction 20.81 ± 0.90. Additionally, there was a decrease in the mean basophil count in the experimental group 5.46 ± 0.78 vs 10.89± 0.96, $p < 0.000$. When compared to the control group, the experimental groups mean chromophobe count increased 57.82 ± 0.96 vs 46.46± 1.09, $p < 0.000$.**CONCLUSION:** Comparing the exposed group to the non-exposed group, there was a decrease in chromophil count and an increase in chromophobe count.

KEY WORDS: *Rats, Ethanol, Pituitary gland, Cell count, Growth*

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INTRODUCTION

It is known fact that 10-15% of all anatomical defects in offspring of humans come from the interaction of several diverse environmental variables during both the prenatal and perinatal stage.[1] Due to a teratogenic substances, the majority of these children have structural defects and growth retardation.[2] According to definition, a teratogen is an agent that has a potential to kill a fetus or permanently alter the structure or function inside the uterus. When exposed to ethanol while in utero, both humans and animals have organ deformity and dysfunction.[3] Alcohol has few positive effects when ingested in modest amounts, but when consumed frequently, it has detrimental consequences on one's health.[4] High alcohol consumption results in poor nutrition, which eventually damages the brain.[5]

Studies in both clinical and laboratory settings have demonstrated that ethanol is a highly strong teratogen and produces "Fetal Alcohol Syndrome".[3] After consumption, ethanol easily crosses the placenta and damages the fetus both directly by building up in the fetal blood and indirectly by building up in the amniotic fluid.[6] By producing toxic metabolites like acetaldehyde, ethanol reduces the ability of placenta to transfer vital nutrients.[7] Low birth weight with both perinatal and postnatal growth retardation is one of persistent characteristic of FAS.[8] Animal studies have demonstrated that short-term blood alcohol levels had more dangerous effects on embryonic development than blood alcohol levels caused by long term alcohol use.[9] The amount of ethanol consumed

and the duration of exposure during development determine the teratogenic insult generated by ethanol.[10] Mouse brain exposed to ethanol during fetal development showed morphological abnormalities in magnetic resonance microscopy.[11]

By blocking PAX6 transcription factor, which is encoded by Pax6 gene[12], ethanol can cause teratogenicity at the molecular level. Peng et al. came to the conclusion that ethanol suppressed 90% of Pax6 expression at concentration as low as 0.3%. Pituitary gland and brain both are impacted by the Pax6 gene.[15,16]

Rat pituitary gland is a midline organ that is situated near the base of brain. It appears in rat between tenth and twentieth day of gestation. The bi-lobed structure has two adenohypophysis lobes with a neurohypophysis in the center Fig. 1. Rathke's pouch develops into an adenohypophysis, with the pars distalis, pars tuberalis and pars intermedia making up the majority of the structure.[16]

It is known that ethanol causes intrauterine growth retardation in fetuses and growth retardation in infants exposed to ethanol, however it is unknown if this change can be objectively assessed in the form of cell count in pituitary gland. The purpose of this study was to ascertain the impact of prenatal ethanol intake via parental route on pars distalis of adult pituitary gland in terms of the cell count.

METHODOLOGY

This research was an experimental, and carried out at the Regional Centre in the Anatomy department of College of Physicians and Surgeons, Pakistan. Sprague Dawley rats were purchased from the National institute of Health NIH for the study. Random sampling was used to gather the samples. Rat female ranged in age from 70 to 120 days and were nulliparous. These female rats were bred in order to reproduce. Rats must meet the selection requirements by being physically normal appearing and healthy-looking. Numbers were assigned to these rats, and measurements of their weights were made. These female rats were let to mate with four male rats in order to reproduce. Spermatozoa on vaginal smears were used to confirm pregnancy and mark the beginning of their pregnancy. After being identified as pregnant, mother rats were divided into two groups with an equal number of mothers, i.e. 8 pregnant rats in control group 'A' and 10 pregnant rats in experimental group 'B'. Pregnant rat in group B received intraperitoneal injection of 20% ethanol solution in normal saline at predetermined times throughout the day 4.44ml/kg/day was the dosage given. [15] .

In control group in a similar manner, intraperitoneal injections of normal saline were given to a mother rat. Only male pups were used in this research, which begin with their birth and continued through their 70th day. At this point, 30 adult male rats were chosen at random from each group and put to death with chloroform. Stereomicroscope was used to dissect out pituitary glands.

The samples were given numbers. The specimen were immersed in Helly's formol, a fixative, for 12 to 20 hours at a low temperature. Then was paraffin embedded. Slides were created in coronal orientation that were six micrometer thick. After that, the periodic acid Schiff-orange GPAS-OG procedure was used to stain the slides[17]. Ten evenly spaced sections were chosen from the total number of sections made for each specimen. Seven fields were chosen, three in each side of the adenohypophysis posterior, middle and anterior and one in the center of the two halves Fig.2. A graticule attached to the microscope's ocular was used to count each type of cell at high magnification 100X. Each group's mean were computed.

RESULTS

In this study we found that group A's mean acidophil count was larger than group B's mean acidophil count. It was discovered that the two's cell count difference were statistically significant. In the pars distalis of the pituitary gland, in Group B the number of acidophils per unit area 10000μ^2 was 24.75% of the total cell counted while it was 40.05% in the control Group A Fig.3

In addition it was also found that the mean basophil count was greater in Group A as compared to the Group B, in the pars distalis of pituitary gland. The number of basophils per unit area 10000μ^2 was 6.49% of the total cell counted in group B while it was 10.44% in the control group A Fig.3

Our examination of pituitary gland cell counts for chromophobes revealed that Group B had a greater number of chromophobes than Group A in the pars distalis of the pituitary gland. In the Group B, the mean of chromophobes per unit area 10000μ^2 was 68.76% of the total cell counted Fig 3, whereas in the Group A, it was 44.54% Fig 4 with p-value < 0.000, this difference *was statistically significant Table-1.*

Table 1: Pituitary gland cell counts for chromophobes in different groups

Parameters	Acidophils	Basophils	Chromophobe
Mean cell count for Group A control ± SE	46.93± 0.71	10.89± 0.96	46.46± 1.09
Mean cell count for Group B Experimental ± SE	20.81± 0.90	5.46± 0.78	57.82± 0.96
p-value	P<0.000	P<0.000	P<0.000

FIGURES

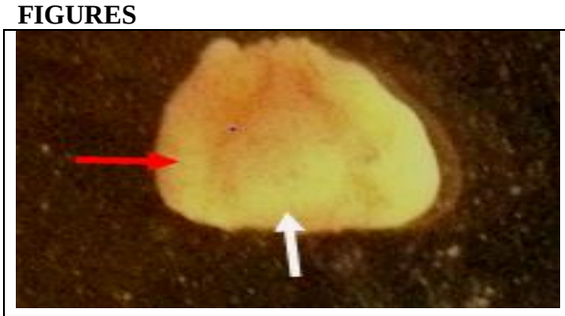


Fig 1: Stereomicroscope image of Pars nervosa white arrow and pars distalis Red arrow.

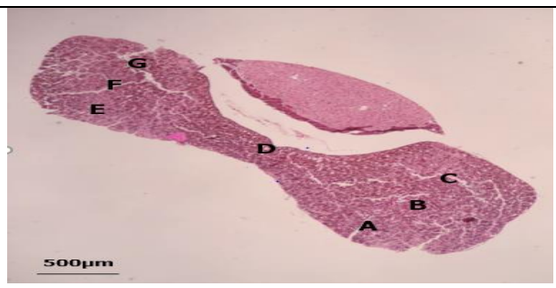


Figure 2: Rat pituitary gland photomicrograph with fields counted. A, B, C of one lobe and E,F,G of the other lobe, and D the center region between the two lobes of the pars distalis indicate the anterior, middle, and posterior portions, respectively

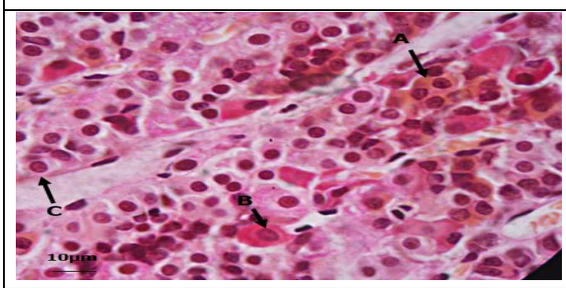
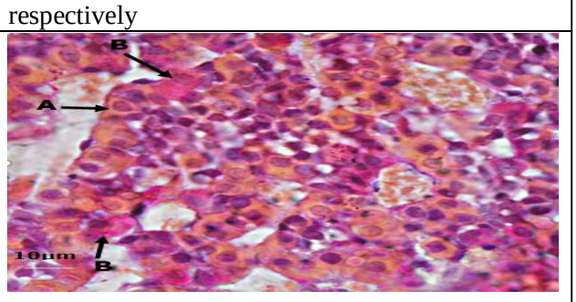


Figure 3: Adult rats in experimental group B's pituitary gland coronal section showed a higher concentration of chromophobes C. There are also few acidophils A and basophils B. Stains from PAS-OG. 10µm Scale bar, 100 x magnification



Figures 4: Adult rats in Control Group A's pituitary gland's coronal section, displaying an increased amount of acidophils A and basophils B. Stains from PAS-OG. Magnification is 100x and Scale bar is 10µm,

DISCUSSION

PEE hinders prenatal growth, which increases fetal mortality and morbidity. Studies on animals as well as on humans have supported ethanol's teratogenic effects. One of ethanol teratogenicity processes involves interfering with the molecular controls on the development of numerous organs, such as pituitary gland.[15] The pituitary gland expresses the Pax6 transcription factor, and its normal expression is necessary for the gland to develop. Somatotrophs and lactotrophs number are significantly decreased in Pax6 homozygous null mice.[15] Research has also demonstrated that, in comparison to controls, rat fetuses exposed to ethanol during gestation have lower serum levels of the growth hormone GH. The anterior pituitary's somatotrophs release GH. [18]

Out of the total number of chromophils, the acidophil count was of particular importance in this study. However, it was discovered in this experiment that the basophil and acidophil counts were both decreased. Pax6, a gene that encodes a transcription factor, is one of many genes involved in the formation of the pituitary gland. It was determined that Pax6 gene expression is crucial for mouse and zebrafish. [19] At the embryonic day 9 and throughout the gestational day 18, the Rathke's pouch expressed Pax6 gene. Alcohol is known to cause microcephaly and growth retardation in dose dependent way, and that effect results from effecting how several genes express themselves which in turn regulate brain development such as Pax6. [15] Alcohol inhibits 90% or more of the Pax6 gene's expression, which results in its teratogenic effects in just 0.3% of concentration. It's possible that ethanol injection to expecting mothers prevented Pax6 gene expression, which in turn decreased Pax6 transcription factor. Reduced Pax6 expression may have contributed to a decrease in number of acidophils in the pars distalis. According to a study that found mice with deleted Pax6 genes lacked LHβ expressing cells in their pituitary glands, the basophil count also decreased. [20, 15] As a result, these cells may be among those that ethanol's action on the Pax6 have blocked. Chromophobe cells are a diverse group that includes all degranulated cells as well as stem cells or undifferentiated progenitor cells. [21] In our study, it was discovered that there were more chromophobes in the central group in the anterior and posterior portions of each of pars distalis lobe. In the experimental group, more chromophobes were discovered in the same areas and their higher quantity may be related to a comparable decline in chromophils. This study is consistent with research done on day 5 rat pups that were prenatally exposed to ethanol. [22] We have demonstrated the structural underpinning of functional deficit caused by prenatal ethanol exposure through our investigation. This research can serve as a strong point for the biologist who wish to address

issues caused by pituitary dysfunction in the future.

The work was hindered by the inability to measure Pax6 expression on the pituitary gland exposed to ethanol during pregnancy.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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AUTHORS' CONTRIBUTIONS: All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST: No competing interest declared.

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CONCLUSION

When ethanol was administered during pregnancy, it slowed down the pituitary gland's growth and development, as demonstrated by a transition in the pars distalis of pituitary from the chromophils to the chromophobes.

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