ORIGINAL ARTICLE

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The Effect of Prenatal Ethanol Exposure on Volume of Pars Distalis of Pituitary Gland in the Rat Pups

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ABSTRACT

Objective: To determine the effect of prenatal ethanol exposure (PEE) on the volume of pars distalis of pituitary gland in the male rat pups.

Methodology: For breeding, sixteen female Sprague Dawley rats were obtained by random sampling method. They were allowed mating with four male rats and divided into control group A and experimental groups B. Experimental group (B) rats were injected intraperitoneally with 4.44ml/kg ethanol while control group (A) with same amount of normal saline from gestational day 10 (GD 10) to gestational day 18 (GD 18). Both groups were given standard laboratory conditions for normal completion of their gestation and were allowed to deliver spontaneously. Only male pups were included in this study. Pups were reared till fifth day postnatally. Pituitary glands were removed and stained with Periodic acid Schiff-orange G (PAS-OG) stain for histological study. Area of pars distalis of pituitary gland was measured using image-J. The area was multiplied by thickness of the section to get the volume of pars distalis of pituitary gland.

Results: Mean volume of pars distalis of pituitary gland was reduced significantly in experimental group (0.085± 0.010 mm3) as compared to control group (0.122±0.005 mm3) (p- value 0.002).

Conclusion: Prenatal ethanol exposure causes significant reduction in volume of pars distalis of pituitary gland in the male rat pups.

Keywords: Ethanol, Pars distalis, Pituitary gland, Rat pups, Volume

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INTRODUCTION

Ethanol is one of the greatest risk factor for morbidity worldwide, with the highest risk in middle income countries.[1] About 4 % of total expires worldwide in a year, are ascribed to ethanol, greater than the expiries caused by HIV / AIDS, Violence or tuberculosis.[1] Lemoine and colleagues in 1968 and Jones et al in 1973 noticed a syndrome in infants of alcoholic mothers which was labeled as "fetal alcohol syndrome" (FAS).[2] Since clinical identification of FAS in 1973, it has progressed from an unacknowledged condition to a principle public health concern. The effect of prenatal ethanol exposure (PEE) on the outcome of pregnancy depends on the quantity and pattern of ethanol consumption.[3]

Low birth weight in infants was identified as the most consistent features of PEE along with pre and postnatal growth retardation.[4] Animal studies has revealed that embryos exposed to elevated blood alcohol concentrations (BAC) over comparatively short periods of time, are more at risk as compared to those of uninterrupted drinking patterns.[5]

The precise mechanism of Ethanol teratogenicity has not been fully elucidated, but the type of damage induced depends on the quantity and developmental timing of exposure, beside other maternal and genetic factors.[6] Studies have shown that one of the mechanisms by which alcohol exerts its harmful effects is through inhibition of expression of Pax-6 gene.[7] Peng et al observed that only 0.3% concentration of alcohol lead to 90% inhibition of expression of Pax-6 gene in Xenopus embryo.[8] PEE has also resulted in reduced expression of Pax 6 transcription factor encoded by Pax 6 gene in rat brain.[9] Normal Pax 6 gene expression is essential for neural[10], pancreatic[11], ocular[12] development as well as development of dorsoventral axis of

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pituitary gland.[13]

Abnormalities of pituitary gland have been recognized on autopsy examination in individuals exposed to ethanol prenatally.[14] The pituitary gland is located in midline at the base of brain.[15] As far as the development of rat pituitary gland is concerned, it occurs between 10th to 20th day of gestation.[15] Pituitary gland consists of glandular (adenohypophysis) and neural (neurohypophysis) parts.[15] Adenohypophysis is derived from Rathke's pouch and consist of pars distalis, pars tuberalis and pars intermedia.[16]

It is known that PEE causes growth retardation but whether this effect is quantitative in terms of volume of pars distalis of pituitary gland was not investigated previously.

The objective of the study was to determine the effect of ethanol administration prenatally on the volume of pars distalis of pituitary gland in the rat pups.

METHODOLOGY

This experimental study was done at the Department of Anatomy, College of Physicians and Surgeons Pakistan (CPSP) Regional Centre, Islamabad from April 2014 to April 2015. Sixteen Nulliparous female Sprague Dawley rats of ages 70 to 120 days were obtained from National Institute of Health (NIH) Islamabad by random sampling method. These female rats were used for breeding. Apparently healthy female rats without any physical deformity were selected. Rats were numbered, weighed, and housed in cleaned cages. Female rats were mated with four male rats. Vaginal smears were collected on daily basis between 07:00 and 09: 00 AM. Female rats were labeled to be at gestational day zero (G0) by the presence of spermatozoa on vaginal smears. Pregnant rats were kept as one rat in one cage and divided into control (A) and experimental groups (B) consisting of 8 female rats each. Group B was injected intraperitoneally with 20% ethanol solution in normal saline at a dose of 4.44ml/kg/day[15] while Group A was injected intraperitoneally with equal volume of normal saline from G10 to G18 keeping environment same for both groups i.e., 12-hour light-dark cycle, controlled humidity, temperature, and free access to standard laboratory rat food and drinking water. For intraperitoneal injection of ethanol, 24 G needles were fitted to 10cc syringe. Mother rats delivered spontaneously, and 5-day old male pups were selected for the experiment. After being euthanized with chloroform inhalation, Skulls were removed. Pituitary gland was approached by cutting Parietal bones at their posterior, lateral and anterior margins. Flap of thin bones were reflected to expose brain. After lifting of the brain by cutting optic nerves and pituitary stalk, the pituitary gland was exposed lying in Sella turcica. Due to small size of pituitary gland, Stereomicroscope was used for fine dissection and pituitary gland was removed along with Sella turcica of sphenoid bone. The specimens were numbered and fixed in Helly's formol for 12 to 20 hours at 40 C.[15] Specimen were processed for paraffin embedding. Rat pituitary gland is disc shape with two wings of pars distalis.[16] Coronal sections were cut at 6 micrometer (µm) thickness and stained with Periodic Acid Schiff-orange G (PAS-OG) method.[17] Sections were examined at low power objective (4X) for volumetry of pituitary gland.

Area of pituitary gland was determined by employing Image-J which is a public domain, java based image processing software developed at National Institute of Health (USA), released in 1997, available at http://www.rsb.info.nih.gov/ij/.

Photographic image of all serial sections of pituitary gland was captured through eye piece of microscope at 4X objective power. Scale for the image-J was set. Images of serial sections were uploaded in the computer (Fig 1). The images were then opened in image-J software. By employing image-J, cumulated area of all serial sections of the entire pars distalis of pituitary gland of rat pups were measured in square µm. The cumulated areas of pars distalis of pituitary gland were then multiplied by the thickness of the section to get the volume of pars distalis of rat pups in cubic µm. The data was analyzed statistically with the Statistical Package for Social Sciences (SPSS) computer software program, version 21. Student t-test was used for analyzing quantitative data and expressed as means ± SEM. A p-value of ≤ 0.05 was considered statistically significant.

The pituitary gland of rat pup sections was photographed through the Olympus binocular microscope at 4X lenses for serial sections using a Nikon SLR camera. Scale bar was drawn by taking two photographs of the same microscopic field and magnification from two eye pieces of the microscope, in one of which ocular micrometer had been fitted. The picture which was captured through the eyepiece with the ocular micrometer showed the image of the scale also, superimposing on the image of the section. A line was drawn parallel to the scale on the picture in a MS word document. The number of divisions covered by this line was noted and converted into micrometers. The ocular micrometer scale divisions had already been calibrated in micrometers with the help of stage micrometer at each magnification. With this, a scale bar of known length was obtained and pasted on picture 2 which was captured through eyepiece without the ocular micrometer.

Comparison of mean volume of pars distalis of pituitary gland (cubic millimeter) of control group A with experimental group B

Parameter	Control A pups n=26 Mean ± SD	Experimental B Pups n=23 Mean ± SD	p- value
Volume of pars distalis of pituitary gland	0.122±0.005 mm^3	0.085± 0.010	0.002

n = Number of specimens SEM = Standard error of mean* = statistically significant

RESULTS

In the male rat pups, the mean volume of Pars distalis of pituitary gland of experimental group B (Fig.1) was significantly reduced as compared to in control group A (Fig.2). (Table).

In addition it was also noted that there was an obvious decrease in the cellular content of pars distalis in the experimental group as compared to the control group (Fig.3).

FIGURES

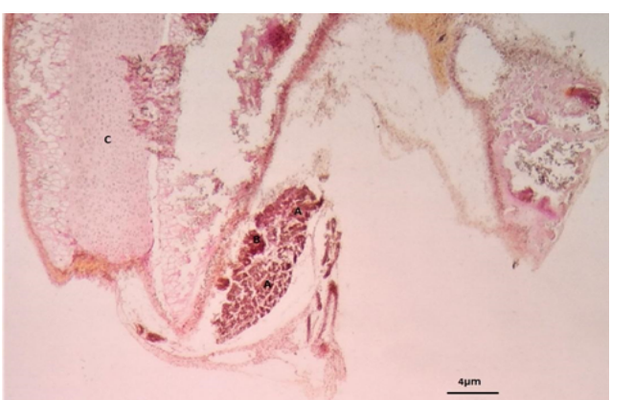

Figure 1: Coronal section of pituitary gland of rat pup of experimental group B showing Pars-distalis (A) and Pars nervosa (B) of pituitary gland and sphenoid bone (C). PAS-OG staining. Scale bar=4µm, Magnification=40.

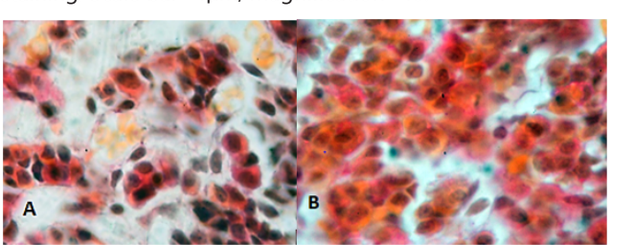

Figure 3: Comparison of cell counts in experimental group (A) and control group (B) of rat pups. Number of cells are reduced in experimental group as compared to control group. PAS-OG staining. Magnification=1000.

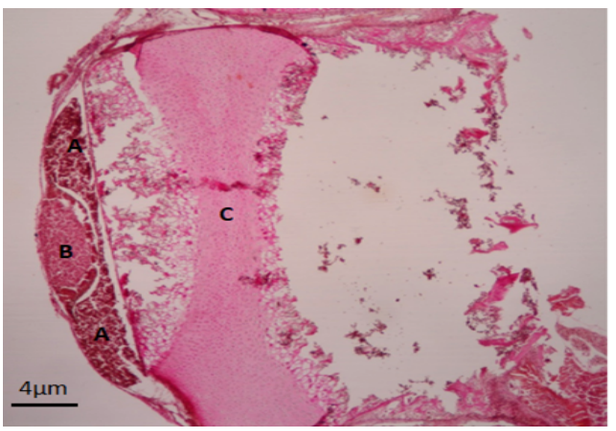

Figure 2: Coronal section of pituitary gland of rat pup of Control group A showing Pars-distalis (A) and Pars nervosa (B) of pituitary gland and sphenoid bone (C). PAS-OG staining. Scale bar = 4µm, Magnification=40

DISCUSSION

Ethanol thorough its high permeability into every organ and tissues of body, can result in organ injury and dysfunction. The severity of ethanol effects on the developing fetus depends not only on the quantity of ethanol consumed and the pattern of ethanol consumption[18] but also the exposure time during gestation.[19] In the present work, with the experimental protocol, high blood alcohol concentration was used via daily ethanol injections from gestational day 10 to gestational day 18, to mimic human FAS in a rat model.[20] This study has shown teratogenic effects of ethanol on the development of pituitary gland by affecting volume of pars-distalis of pituitary gland. This effect could be explained on the molecular basis of development of pars distalis of pituitary gland.

Normal expression of Pax 6 gene is required for the development of pituitary gland[13] as well as other organs like eye[12], pancreas[11] and nervous tissue10. Pax6 is expressed in zebrafish and murine embryonic pituitary.7 Pax6 homozygous mutation led to death of mice before birth. Pax

6 mutated mice had low serum growth hormone (GH) level during embryonic as well as postnatal life, although mRNA for these peptide hormones was present. Alcohol concentration of just 0.3% could reduce 90% of Pax6 expression.[7] Similarly Studies have shown that overexpression of pax6 on alcohol treated radial glial cells rescued them.[21] In an experimental study in which the animals were prenatally exposed to ethanol resulted in methylation of DNA and reduction in growth factors required for the growth of granule cells of cerebral cortex in offspring.[22] Ethanol also exerts its harmful effects by interfering with proteostasis within and outside the cells.[23] More production of abnormal proteins would lead apoptosis of cell organelle and ultimately apoptosis of whole cell thus in turn reducing the cell count.

All these studies suggested that ethanol thorough its inhibitory effects on cells can result in reduced number of cells, which in case of our study leading to reduction in volume of pars distalis of pituitary gland which is most cellular part of pituitary gland.

CONCLUSION

Prenatal ethanol exposure showed inhibitory effects on growth of pituitary gland as shown by decrease in volume of pars distalis of pituitary gland of rat pup.

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