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### Effect of cyclophosphamide in histological features of cerebellum in chick embryo

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#### ABSTRACT

Cyclophosphamide is an Alkylating Agent and it comes under the category of Nitrogen Mustards, which is used commonly in the treatment different types of Cancers or Carcinomas or Neoplasms (Goodman and Gilman 9th edition 1996). It was synthesized and Introduced in the year 1854 and its properties were elaborately observed and described by 1887. Cyclophosphamide is administered in the treatment of Acute Lymphoblastic Leukemia, Chronic Lymphocytic Leukemias, Hodgkin's Lymphoma, Non-Hodgkin's, Lymphoma, Multiple Myeloma, Neuroblastoma, Breast Cancer etc. It is also a potent Immuno Suppressive Drug. It has been used for the Control of Organ Rejection after Transplantation, Wegner's Granulomatosis, Rheumatoid Arthritis, Nephrotic Syndrome in Children. In the present study fertilized eggs were administered with Cyclophosphamide and histological features of cerebellum were studied after 20 days. Cyclophosphamide cytotoxicity results in reduction of multiplication of cell activity associated with malformations and embryonic death. Injection of the drug causes decrease of mitotic activity which produces deformities.

**Keywords:** Cyclophosphamide, Cerebellum

#### INTRODUCTION

The study of the Cerebellum in Chick embryo (*Gallus gallus*) is very important because it controls physical activities, maintenance of the balance of the organism, regulation of muscle tone (1, 2). The

chick brain consists of three main parts cerebrum; cerebellum and medulla oblongata. The chick has large cerebral hemispheres and small cerebellar hemispheres (3). There are no significance differences that occur in the central nervous system of the birds compared to that mammal (4). The

cerebral hemispheres were separated from cerebellum by a transverse fissure (5). The cerebellum in chick embryo of 20 days age consists of two components, gray matter and white matter. The gray matter is situated externally while the white matter is situated internally (6). Also cerebellum consists of cortex and medulla. The cortex consists of three layers molecular layer, purkinje cell layer and granular layer (7).

Considering the Multifunctional Property of Cyclophosphamide, the present study was undertaken to observe and elucidate the changes in the Cerebellum after administration of Drug. The changes were considered in relation to the anti-histogenesis or anti-mitotic activity of Cyclophosphamide by administering the drug into Fertilized Eggs. An attempt has been made to observe if the Drug passes through Placental Barrier leading to Malformations of Foetus. Such a study will help in treating Cancer Patients who are Pregnant. Cyclophosphamide has been experimented by few Scientist's on Laboratory animals like Chick Embryos etc. and their observations were noted.

## **MATERIAL AND METHODS**

### **Selection of eggs**

Well developed, mature and healthy fertile eggs are selected from the breeders that are white leg horn (*Gallus gallus*). Excessively large or small eggs, cracked or thin shelled eggs are avoided because they will have difficulty in retaining moisture which is needed for proper chick development. Penetration of microorganisms increases in cracked eggs.

Eggs should not be washed or wiped with clean cloth as it removes the protective coating and promotes the entry of microorganism. Rubbing and washing also serves to force disease organisms through the pores of the shell.

### **Incubation of eggs**

Done for a period of 21 days. The temperature should be

101 degree Fahrenheit for first week

102 degree Fahrenheit for second week

103 degree Fahrenheit for third week.

Optimum growth for most of the species requires a relative humidity of 60% until eggs begin to pip, after which the relative humidity should be raised to 70%

The humidity is maintained inside the incubator is maintained by placing an open pan of water with suspending a piece of cloth from the water, proving wick action.

### **Administration of cyclophosphamide in to intact chick embryo**

Five eggs were kept as control and cyclophosphamide was injected into other set of five experimental eggs. A small hole over the broad end of the experimental egg was made using 22-gauge needle. 0.25 micrograms of cyclophosphamide was injected into the egg after 48 hours of incubation. It was done with an insulin syringe. Following drug administration; the holes were sealed with molten wax after which the eggs were placed back into the incubator.

### **Processing and staining**

After 20 days of incubation the eggs were broken and the embryo was collected and fixed in 10% formalin solution for 48 hours. The brain tissue was separated, processed and stained with Haematoxylin and Eosin stains.

The slides were studied under the simple microscope and various features were identified.

### **Data analysis**

The data is analyzed statistically using SPSS software (version 17.0)

## **RESULTS**

Cyclophosphamide administration resulted in a dose dependent massive reduction in number of cells of cerebellum as compared to the number of cells of cerebellum from control. Cyclophosphamide induced cytotoxicity was presented by reduction of cell-cycle resulted in an overall decrease of multiplication activity of nervous tissue. It was clearly associated with reduction of number of cells in all layers of cerebellar cortex.

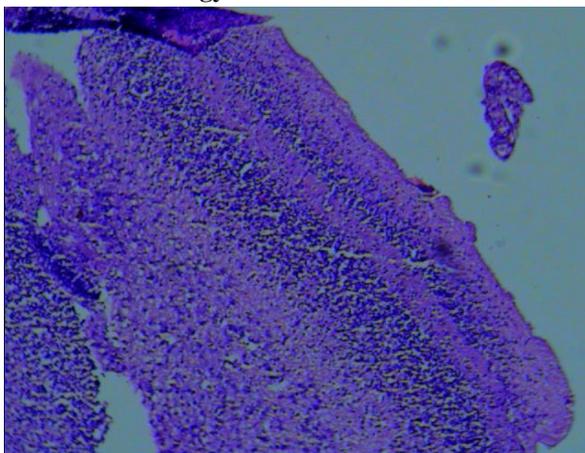
### Normal embryo



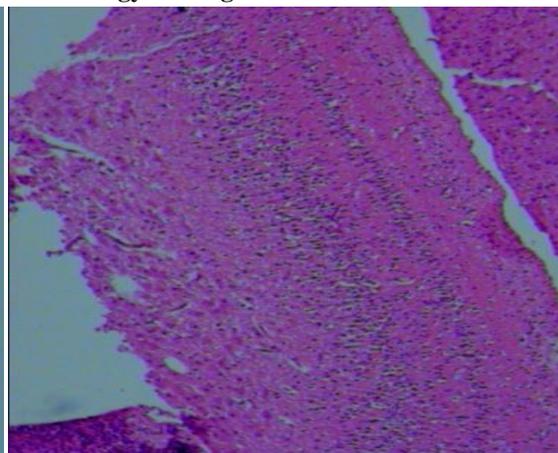
### Undeveloped embryo after Treatment with cyclophosphamide drug



### Histology of normal Cerebellum



### Histology of drug treated cerebellum



## DISCUSSION

The loss in the cellularity in all the layers of cerebellum could be attributed to two factors: (1) a decrease in multiplication of cerebellar cells and (2) induction of cell death in the cerebellar cells of Cyclophosphamide treated chick embryos. The results of the present study endorse both the possibilities. Cerebellar cells obtained from Cyclophosphamide treated chick embryos upon incubation in vitro showed reduced multiplication ability (cell number) as compared to cerebellar

cells of untreated chick embryos. The cerebellar cells of chick embryos

Obtained from Cyclophosphamide injection showed an increased population of cells with typical apoptotic morphology.

The main effect of cyclophosphamide is due to its metabolite phosphoramidate mustard which is formed in cells that have low levels of ALDH (Aldehyde dehydrogenase). The metabolite forms DNA crosslinks between and within the DNA strands at guanine N7 positions which result in cell death. The toxicity is greatest during the S or DNA synthetic phase of cell cycle.

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- [5]. Dyce, K.M;Sack, W,O and Wewsing, C. JG. (1987).Avian Anatomy Text book of veterinary anatomy pp: 772-797. Fig(8)Transverse section medulla oblongata A-the first zone B-the second zone C-the third zone 800x H&E Fig(6) Transverse section of the cerebellum Awhite matter B- purkinje cells C-granular layer 800x H&E Fig(7) Transverse section of medulla oblongata A-the first zone B-the second zone C-the third zone 300x H&E J.Thi-Qar Sci. Vol.3 (3) Aug./2012 05
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