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A study of the effect of dexamethasone on the neural tube development in the Swiss albino mice embryos

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***Abstract*---**Current study includes an investigation of the effects of Dexamethasone (Dex) drug on neural tube development. A total of 60 pregnant mice were allocated into four groups at random, with 15 mice each group, While the control group received injections of a Normal Saline 0.9 %, each group's members were given a specified dose of Dexamethasone drug and at different times. All of the animals received their doses through tail intravenous injection at a specific periods of pregnancy then the embryos isolated at specific embryonic days . The results of various doses of dexamethasone reveal that the number and concentration of doses have an increasing of negative effects on the neural tube development of mouse embryos, neural tube abnormalities (NTDs), Spina bifida, a convoluted body and tail, spina bifida cystica, and a porous longitudinal section in the neural tube, the general external morphological characteristics of embryos that were also influenced by Dex. Dex has a harmful effects on the development of the neural tube, particularly when the dexamethasone administered over an extended period of time and in many dosages.

***Keywords*---**dexamethasone, neural tube, albino mice embryos.

Introduction

The body naturally secretes small amounts of the steroid hormone cortisol, this natural steroid is essential for the body's equilibrium, and its synthetic analogs

are widely utilized to treat a wide range of conditions(1).Dexamethasone (Dex) or sirolimus , one of a synthetic long-acting glucocorticosteroid hormones, one of the most commonly recommended medications for the treatment of inflammatory disturbances such arthritis, asthma, kidney diseases, edema, and redness of the skin. Additionally, it is crucial for the growth and maturation of several fetal tissues, including the adipose tissue, gut, liver, lungs and skeletal muscle in order to prepare for life outside the womb (2). Early mouse neural tube patterning occurs between the ages of 7.5 and 9.5, establishing broad domains along the anterior-posterior axis that subsequently give rise to specific functional parts of the central nervous system, including the forebrain (or prosencephalon), midbrain (or Mesencephalon), hindbrain (or Rhombencephalon) and the spinal cord (3) .The central nervous system (CNS), which is made up of the brain and spinal cord, develops from the neural tube (4), the development of the neural tube which occurs in mammalian include two stages primary and secondary neurulation is termed as neurulation (5).These two stages occur at several locations along the embryo's rostrocaudal axis, mostly tail bud, which is positioned beyond the the caudal neuropore, primary neurulation, in contrast to the secondary neurulation, results from the multiplication of stem cells (6). The neural plate first appears at the cranial end of embryo and develops forward a caudal direction, the neural fold is formed as the plate's edges thicken and start to ascend. The neural plate develops from being an elliptical shape to getting a keyhole shape, getting longer, narrower, and longer, cell intercalation in the midline and medial-directed polarized cell motions are responsible for this transformation (4). After conception, the fetus begins to grow, and this growth is controlled by chemical bonds between cells and layers of cells, and this interdependence is controlled by gene expression(7).Deformations signify mistakes made during this process, when wildebeests develop abnormalities, chemical bonding or translational genetic ordering problems occur (8). There are two types of congenital deformities in embryos: internal genetic reasons resulting from gene mutations or defective chromosomes (9). and Teratogens are external variables that change embryonic development, environmental conditions or external factors (EFs), which encountered the uterine and placenta (10)(11)

Materials And Methods

Preparation of the experimental animals

Albino Mice females , type *Mus masculus*, strain Balb /c, weighing 30±2 gm, were used in present experiment. They were obtained from the Biology Department's Animal House at the College of Education for Pure Sciences - University of Thi-Qar. Animals were placed in the room in plastically cages for breeding with metal covers and brushed with sawdust, in the organized and regulated environmental circumstances, Temperatures ranged from 20-24℃ under continuous photoperiod (12 hours of daylight \ 12 hours darkness),to make sure the mice are healthy and disease-free, they were taken to the veterinarian. Mice were kept in clean conditions by changing the sawdust in their cages every two days. They were also provided with enough water and food from local sources, which included grains such 10% animal protein , 20% barley, 25% corn, , 10% powdered milk , salt 1% and 34% wheat, all was ground and combined with a little water and oil to make a cohesive paste (12). When placed in the specified place for the feeding in the

cages for animal breeding, two to three mature females and one mature male were then caged together overnight, then the following morning, the females were checked for the vaginal plug (13). The day of mating is the day zero (D0) of gestation , and the day following is the first day of pregnancy, according to the date of mating previously recorded on the cages (14).

Dexamethasone doses preparation

Different doses of dexamethasone sodium phosphate (8 mg in 2 ml) aqueous solution were administered to the treated mice. The mice received intravenous injections through the tail vein, different concentrations of the drug (Dex) were chosen depending on the therapeutic dosage (8mg⁄70 kg) (11) which is equivalent to 0.1ml per kg (0.002 ml per 25 gm) of the mouse weight, the fifteen pregnant mice in each of the experimental groups received the following pharmacological treatments at different dosages:

1. The first experimental group: was administered a dose of 0.05 mg Dex per 1 kg of body weight .
2. The second experimental group received treatment at a dose of 0.1 mg of Dex per 1 kg of body weight .
3. The experimental group: was given a dose of 0.2 mg of Dex for each 1 kg of body weight.

The mice were injected started on day eight of pregnancy and continuing through day one of delivery. The embryos were isolated when the mice were dissected at embryonic days 11, E13, E15, and E17.

Isolate the mice embryos

To minimize the risk of mouse hair contaminating the dissection, the pregnant mice were washed in 70% ethanol. Using standard surgical scissors, the skin was squeezed, and a little of the lateral incision was created at the midline. To expose the abdomen, above and below the incision, the skin was firmly pulled. and pushed apart in the direction of the head and tail. The abdominal cavity was made visible by cutting the peritoneum while using forceps to hold it. By seizing the uterus below the oviducts and cutting it free along the mesometrium layer , the uterine horn was extracted. By making cuts along the uterine horn between the implantation sites, each embryo was isolated from the uterus and from the others.

The histological study for embryonic tissues

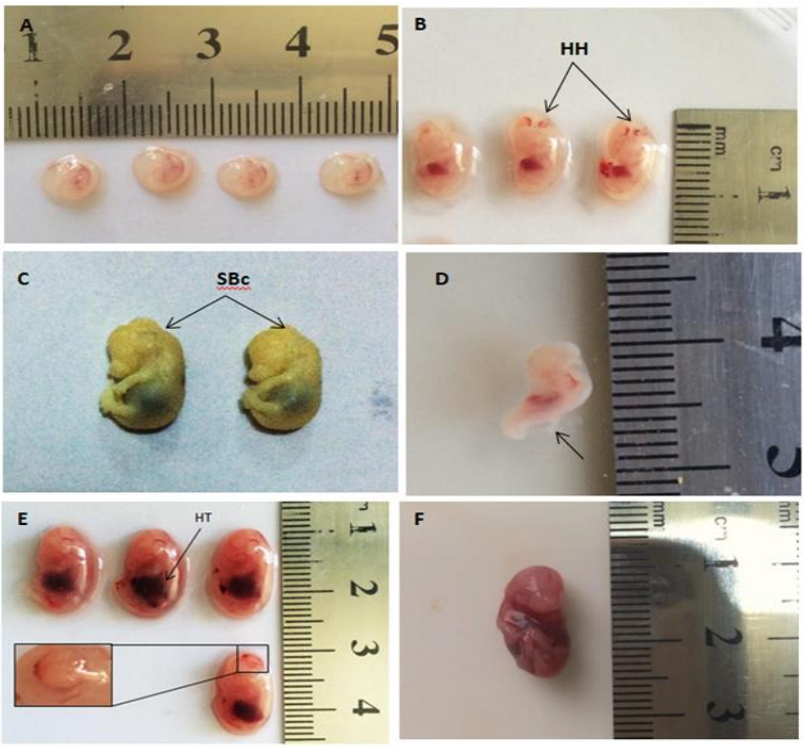
The preparation of solutions and dyes , which was using during this study according to (14). Which includes : Fixation, washing, dehydration, clearing , infiltration , embedding , the trimming and sectioning , staining , mounting then histological slide examination and photography, where the slides were viewed using a microscope (Leica microscope) of Germanic ancestry with several powerful zooms, and then photographs were taken using a camera linked to the microscope.

Statistical analysis

Least Significant Difference Test (LSD) was used to analyze the data, which were reported as mean and standard error (SE) using SPSS software (ver 24.0) for Windows. At $P \leq 0.05$, differences were considered statistically significant.

Results

The morphological defects and various alterations that occurred in the embryos treated with various dosages of Dexamethasone were demonstrated by the findings of the current study. The fetus's body is twisted and resembles a ball or lump of flesh or mass of meat embryonic day 11 treated with 0.2 mg/kg of Dex (Figure1:A) ,Hemorrhage was observed in the head of embryos at E13 was caused by 0.1 mg/kg Dex (Figure1:B), Spina bifida in at E13 embryos treated with 0.2mg \kg Dex (Figure1:C) Trunk torision for embryos at E11 treated by 0.2mg\kg of Dex (Figure1:D) and at P1(0.1mg\kg Dex)(Figure1:I) Tumescence in the head of embryos E15(0.05mg\kg Dex) (Figure1:E), results of current investigation showed that there was trunk curvature, aberrant limb development, and a convoluted neck and tail. at E15(0.2mg\kg Dex) (Figure1:F), opening neural tube E11 (0.2mg/kg Dex)(Fig1:G) curvature of the head to another side with convoluted tail E17 (0.1mg\kg Dex)(Fig1:H) spherical shape embryos E15 (0. 2mg \kg Dex)(Fig1:J) . At the embryonic day 17 (0.2mg\kg) neural tube defects (Fig1:K) convoluted tail E17(0.1mg/kg Dex)(Fig1:L), there was spina bifida cystica at head region E17 (0.2 mg/kg dex) (fig2: J).



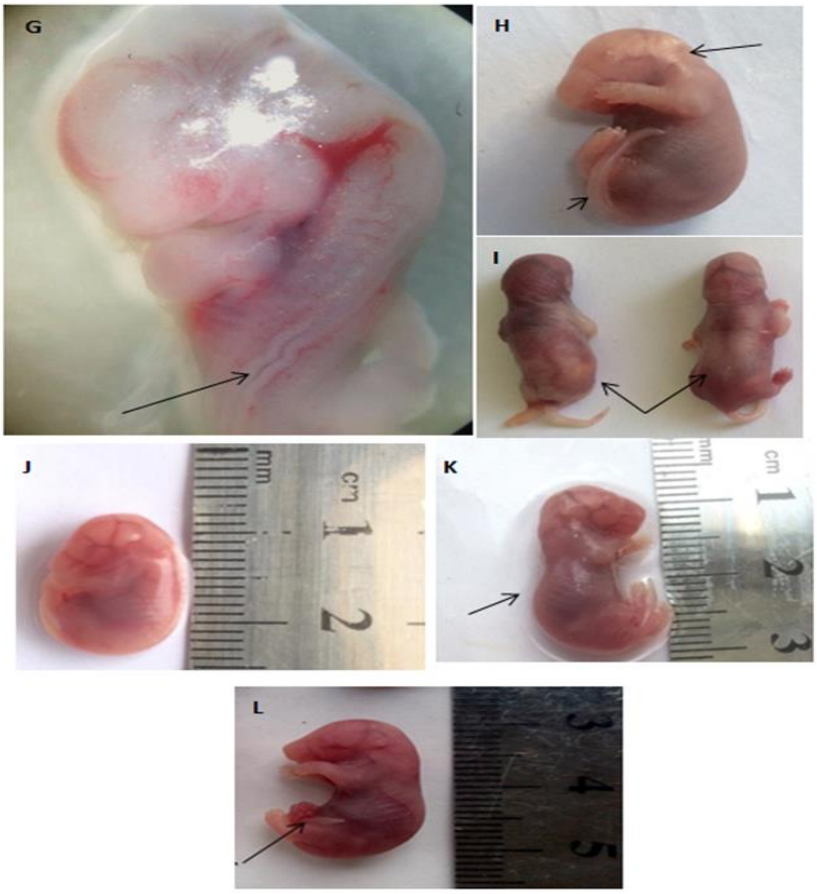


Figure1:(A) Embryos at E11 look as ball of meat, (B) HH: head hemorrhage in embryos at E13/ 0.1mg\kg Dex, (C) SBc: Spina Bifida cystica (E13 /0.1mg/kg Dex), (D) Trunk torsion (arrow)(E11/0.2mg/kg Dex),(E)HT Hypertrophy in the liver magnificent section to brain explain clear spina bifida cystica (E15 /0.05 mg /kg Dex), (F) trunk torsion ,abnormal limbs development with convoluted at the neck and tail (E15/0.2mg/kg Dex), (G) spina bifida (arrow) E11/0.2mg/kg Dex ,(H)curved head (long arrow) convoluted tail (short arrow) E17/0.1mg /kg Dex, (I) trunk torsion (arrow) p1/0.1mg/kg Dex ,(J) spherical shape embryo E15 /0.2 mg /kg Dex, (K) NTD (arrow) E17 /0.1mg/kg Dex,(L) Convoluted tail (arrow)E17 /0.1mg /kg Dex .

Numerous histological alterations to the neural tube were observed in histological sections, including an open neural tube at E13, E11 through treating with 0.1 mg/kg Dex (Figure 2: E&F), spina bifida cystica at E13 when they treated with 0.2 mg/kg Dex(Figure2:G), and an open neural tube with damage to the neural folds at E15 their mothers injected with 0.2 mg/kg of Dex (Figure2:H). There was hemorrhage in the neural tube that belonged to NTDs (Figure2:I), and there was a pore in the longitudinal section of the neural tube at E13 at the dosage 0.1 mg/kg Dex (Figure2:J) ,neural tube is curved to inside E17 at the concentration 0.2 mg/kg of Dex (Figure2:K) ,spina bifida at E11(0.2mg/kg Dex)(Figure 2:L)Spina bifida occurs at E11 (0.2 mg/kg Dex), and the neck area (E15/0.2

mg/kg Dex) has an extra-curved neural tube (Figure2:M). Spina bifida cystica E17 when they treated with 0.2 mg/kgDex (Figure2:N) .

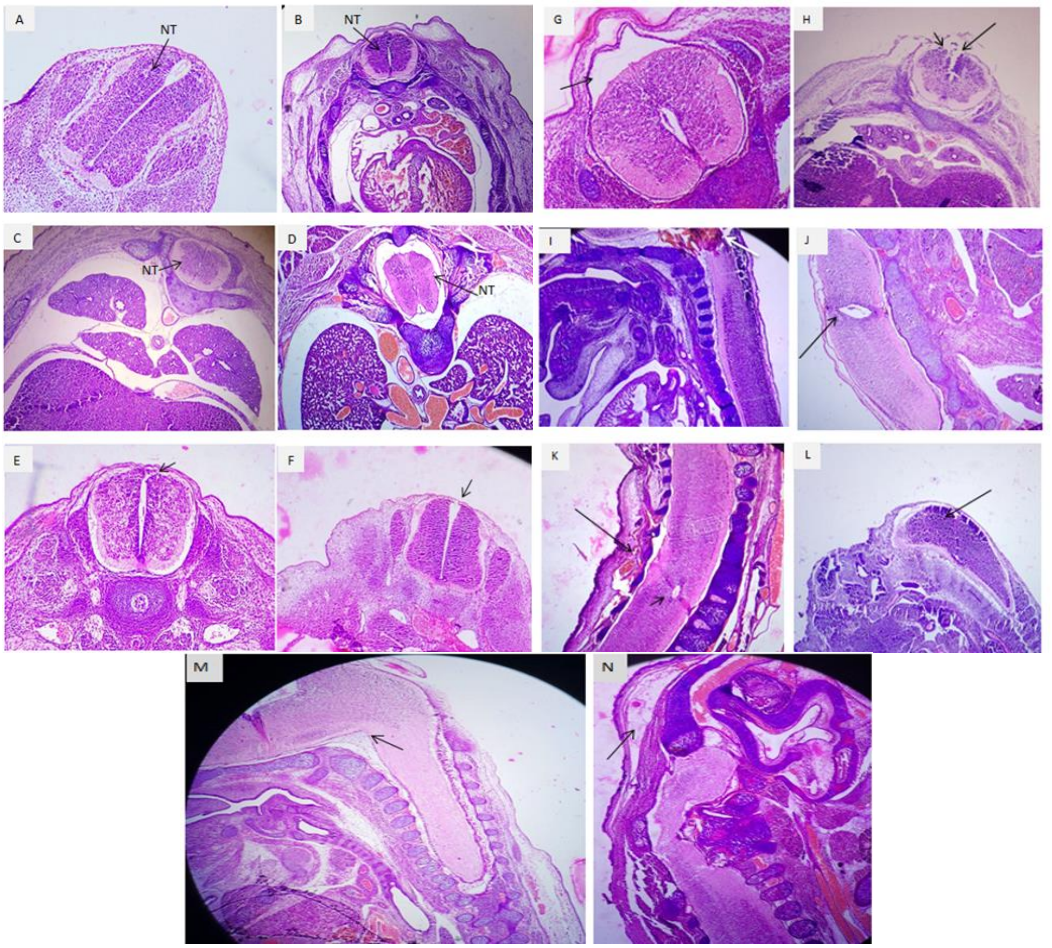


Figure2: (A) E11 control ,(B) E13 control, (C) E15 control, (D) E17 control (NT)Neural Tube , (E) open neural tube (arrow) E13(0.1mg/kg Dex)(H&E 100x),(F) Open neural tube (arrow)E11(0.1mg/kg Dex)(H&E100x),(G) spina bifida cystica (arrow)E13(0.2mg/kg Dex (H&E 40x),(H) open neural tube (long arrow)damage in neural folds (short arrow) E15 (0.2mg/kgDex)(H&E 40x),(I) hemorrhage in nural tube (arrow)E13(0.2mg/kg Dex)(H&E 100x),(J) pore in longitudinal section of neural tube (arrow) E13(0.1mg/kg Dex) (H&E100x),(K)Curved neural tube to the inside (long arrow) pore at longitudinal section in neural tube (short arrow) E170.2 mg/kg of Dexamethasone (H&E 100x),(L) spina bifida (arrow) E11 treated by 0.2 mg/kg of Dex (H&E 100x),(M) curved neural tube (arrow) E15 injected by 0.2mg/kg of Dexamethasone (H&E 100x),(N) spina bifida cystica (arrow) E17 (0.2 mg/kg Dex) (H&E 40x).

Discussion

The cells undergo a metamorphosis process and follow a specific growth program throughout the firstly three months of gestation in humans, which is comparable

to the first week of pregnancy in mice (16). The most crucial period is during the growth and formation of different structures, refers to the period of time during which organs and tissues are most susceptible to changes due to their sensitivity to these influences as they occur during the growth and production of distinct structures (17). the structures changes are influenced by the stage of embryonic development and the concentration of the deformational materials (8).Neural tube malformations are a congenital abnormality of the central nervous system and rank (18). A collection of severe congenital neural system malformations known as NTDs are caused by the failure of closing of the neural tube during neurulation (19), the curving of the neck, especially at E15 treated with 0.2 mg/kg of Dexamethasone , which forms spherical-shaped embryos, and the curvature of the head toward the chest of embryo , the nervous system malfunction was used to explain the deformities in the head region, and this was confirmed by (20). This study also described how dexamethasone treatment during pregnancy altered the development of synapses in the central nervous system (21).

Utilising dexamethasone during pregnancy, especially during organogenesis, results in a defect in the way the neural folds close, which causes neural tube defects (NTDs) (12) This is consistent with what found in the current study. As a result of the deformation of the vertebrae which ultimately leading to a swelling (or edema) in the spinal cord on the dorsal surface under the body covered with skin, (22) explained the swelling or the edema in the dorsal region of the embryo as result of a rare malformation in the spinal cord termed as Meningoen cephalo cells (MCCs). Thus, the Neural tube defects and deflection or the curvature in the embryo trunk can led to formation of ball-shaped or a spherical-shape Embryos at E15.

At embryonic day 8.5, the hindbrain/cervical boundary is where the mouse neural tube initially fuses, and at embryonic day 10, the neural tube completely closes (23). The development and differentiation of neurons can be inhibited in neonates exposed to glucocorticoids (24, 25, 26). Neural tube defects (NTDs) are developmental malformations caused by failure of the neurulation process, which develops the neural tube, the embryonic origin of the brain and spinal cord (27) and can cause spinal anomalies that delay the closure of the posterior neuropore (28). Anencephaly and spina bifida result from incomplete closure of the neural tube at the rostral or caudal ends (29). Glucocorticoids are hypothesized to cause teratogenic effects (30).In several species, such as the mouse (31), glucocorticoids, including Dexamethasone, are known to be animal teratogens. Hemorrhage in the neural tube may be associated to the extremely convoluted nature of the neural tube, which caused damage to the neural tube tissue. Dexamethasone can cause teratogenic effects in certain animal species, such as rabbits' neural tube defects (32). In both the morphological and histo-morphological tests, this study detected failure of neural tube closure. Dexamethasone may be the cause of the inhibition of cell proliferation, which delays and prevents a normal neural tube closure.

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