Study the Effect of Dexamethasone on the External morphology features at different Embryonic Developmental stages in the Swiss Albino Mice Embryos.

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Abstract

The current study was conducted for the period from September 2015 until August 2016 in college of Education for Pure Sciences - University of Thi- Qar, this study aimed to fallow the changes on the External morphology features at different Embryonic Developmental stages when the pregnant mice treated with different doses of Dexamethasone (Dex). Use In the current study, sixty pregnant mice were randomly divided into four groups and each group of 15 pregnant mice. Given the members of each group specific dose of Dex and at different time periods, while the control group injected with a solution of Normal Saline 0.9%, all animals received doses used by tail intravenous injection until the end of the time periods specified. The treatment of animals under the same conditions were determined dose based on body weight, according to what is stated in the pharmaceutical constitutions. The results of statistical analysis at the level of probability ($p \leq 0.05$) to different doses Dexamethasone show there is an negative effects on mice embryo's body weights and the lengths increase with increasing of number and doses concentrations. using of different doses of Dex showed various changes on general external morphological features and congenital malformation in embryos of treated mothers included: Death of embryos , Letter C Shape Embryos , Head hemorrhage , placenta damage , Neural Tube Defect, Trunk Torsion, curved head , Brain hypertrophy , Liver Hypertrophy , swelling , Short nostril and limbs, Convoluted Tail, Oedema ,Wrinkled skin, absent fingers and eyelids, Crooked pave, Short toes, Seal legs appearance , Tail congestion , Bulging eyes.

Key words: Dexamethasone, Albino Mice Embryos.

The Abbreviations: Dex:Dexamethasone ,E: Embryonic day ,P: Post-natal day. NTD :Neural Tube Defect.

دراسة تأثير الديكساميثازون على الخصائص المظهرية العامة لمراحل تطورية مختلفة في اجنة الفئران البيضاء

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الخلاصة

اجريت الدراسة الحالية في مختبرات كلية التربية للعلوم الصرفة وللفترة من ايلول 2015 الى آب 2016 .هدفت الدراسة الحالية الى دراسة تأثير الديكساميثازون (Dex)على الخصائص المظهرية العامة لعدة مراحل تكوينية مختلفة في اجنة الفئران البيضاء. أستخدم في الدراسة الحالية ستون فأرة حامل تم توزيعها عشوائياً الى اربع مجاميع كل مجموعة تضم خمس عشر فأرة حامل . اعطي افراد كل مجموعة جرعة محددة من Dex وعلى مدد زمنية محددة بينما حقنت افراد مجموعة السيطرة بالمحلول الفسلجي 0.9% , تلقت جميع الحيوانات الجرع المستخدمة عن طريق حقن الوريد الذنبي حتى نهاية المدد الزمنية المحددة. تم معاملة الحيوانات تحت الظروف نفسها وتم تحديد الجرعة بالاعتماد على وزن الجسم وحسب ماورد في الدساتير الدوائية . اظهرت نتائج التحليل الاحصائي عند مستوى احتمال ($p\leq0.05$) لجرع مختلفة من الديكساميثازون ان هناك تأثيرات سلبية على اوزان واطوال الاجنة تزداد بازدياد عدد وتراكيز الجرع.استخدام جرع مختلفة من الديكساميثازون اظهرت تغييرات في الخصائص المظهرية الخارجية العامة و تشوهات خلقية في الاجنة للامهات المعاملة تضمنت : موت الاجنة , الاجنة بشكل حرف C , نزيف الرأس, تلف المشيمة, عيوب الانبوب العصبي ,انحراف الجذع , ,تضخم الدماغ ,تضخم الكبد , تورم , قصر الانف والاطراف ,التفاف الذيل , استسقاء, تجعد الجلد ,انحناء الرأس , انعدام الاصابع واجفان العيون ,انعقاف الكف ,قصر اصابع القدم , مظهر ارجل الفقمة ,نزيف في الذنب, انتفاخ العيون .

Introduction

Dexamethasone (Dex) a synthetic long action glucocorticosteroid hormone , is one of the most widely prescribed drug for the treatment an inflammatory disturbance such as adrenal hormone insufficiency , swelling , arthritis, redness of skin , asthma and kidney disorders. Dexamethasone has been used to develop the effect of anti-cancer drugs (Shi *et al*.,2014). It is also been used to reduce the risk of neonatal respiratory distress syndrome (RDS).

Fetal growth begins after fertilization and continuation of this growth regulated by chemical bonding between cells and layers of cells this interdependence is regulated through gene expression (Meteyer, 2000).

Deformations represent mistakes in this process. The chemical bonding or translation genetic orders errors appear during the wildebeest form Abnormalities (Pastuszak,2001). Congenital malformations in the fetus

appears as a result of two factors: internal genetic causes result from mutations in a gene or chromosome abnormalities (Kraita *et al.*,2002**) ,** External factors (EFs) are environmental conditions experienced by the placenta and uterus which may lead to birth defects, (EFs) called Teratogen which changing the fetal growth (Alt, 2000) (Abdul-Fattah,2007) it's include: radiation (Pastuszak, 2001),chemicals like Methylmercury (Alt,2000) drugs such as Cyclophosphamide Aljowali,2005) excessive smoking and alcohol , some virus infected parasites such as Syphilis,Toxoplasmosis,Rubella and ADIS,A pathogenic microbes such as E.coli in Amniotic Fluid (Abdul-Fattah, 2007) .

Influence of deformed material as its concentration, and effect the resulting evolution of patients, are determined by the sensitivity and the generator as well as growing stage of the target tissue. (O'Day, 2004), Because of the similarity between rodent and human in terms of fetal development and especially white mice and rat scientist focused their study on different technique, they often classify congenital malformations.

Methods .

1-Experimental animals preparation.

In the present study, Female Albino Mice,type *Mus masculus* the strain Balb /c ranged in age between 11 to 12 weeks,30 ± 2 gm obtained from the Animal House return to the Biology Department - College of Education for pure science / Thi- Qar University,Mice were put in the room in plastic cages breeding with metal lids and Brush the cage with sawdust ,in the organization and controlled environmental conditions at the constant Photoperiod(12 hour day/12 hour night)cycle, ventilation, temperature ranged between 20-24 c,The mice were took to the vet to ensure their health and they are free from disease. Mice were kept under cleanliness conditions of the cages through a change sawdust once every two days, Animals were given a sufficient amount of water and food locally source (Wheat 34% , barley 20% ,corn 25% ,animal protein 10% , powdered milk 10% ,salt 1% all material grinding and mixing with some oil and water until they become a paste coherent) (Tayfur, 2013) and put in the designated place for the food in the cages, Animal breeding , then two mature female was caged together with one mature male overnight and in the following morning the female were checked for the vaginal plug (Saadalla ,2009),Date of mating was written on the cages ,the day of mating is Day zero (D_0) of pregnancy and the day after is the first day of pregnancy (Bogumil, Wlodarczyk, & Minta, 2000)

2- Dexamethasone preparation.

Dexamethasone sodium phosphate (8mg \2ml) aqueous solution was used to treat the experimental animals in different doses. The mice were intravenously injected via tail vein. The different concentration of drug were chosen according to therapeutic dose (8m to 70 kg) (Tayfur, 2013) ,that equivalent to 0.1ml \1kg (0.002 ml \25 gm) from the mice weight. The experimental groups consisting of fifteen pregnant mice for each group, they treated with different doses of drug as follows:

- The first group: treated with the dose 0.001 mg for each 25 gm from the mouse body weight (Equivalent to0.05 mg for each 1kg from the body weight).
- The second group: treated with the dose 0.002 mg for each 25 gm from the mouse body weight (Equivalent to0.1 mg for each 1kg from the body weight).
- The third group: treated with the dose 0.004 mg for each 25 gm from the mouse body weight (Equivalent to 0.2 mg for each 1kg from the body weight).

The mice were injection starting from the eight day of gestation between the day and another to the first day of birth. The mice were dissection at Embryonic days 11, 13, 15, 17, and first day of birth. The embryos were isolated

3-Isolate of the mice embryos.

After the blood was collected from the pregnant mice, the pregnant mice were soaked in 70% ethanol to diminish the risk of contaminating the dissection with mouse hair. The skin was pinched and makes a small lateral incision at the midline with regular surgical scissors. The skin was Holed firmly above and below the incision and pulled apart toward the head and tail to expose the abdomen. The peritoneum was grasped with forceps and cut to expose the abdominal cavity. The reproductive organs located in the dorsal region of the body cavity, two uterine horns, the oviduct and the ovaries. The uterine horn was removed by grasping the uterus below the oviduct and cut it free along the mesometrium. Each embryo was separated by cutting between implantation sites along uterine horn.

The muscular uterine lining was grasped by sliding forceps between the surrounding muscle layer and enveloped decidua tissue. The muscle layer was ridded and a portion of the decidua exposed then the embryo shelled out by using the tips of forceps and removed the embryo. The length and weight of an embryo was note down then we examine the malformation and

the changes and record it then take photograph by using camera photography for it ,then embryos were kept in container contain 10% formalin .

The Results and Discussion

During the first three months of pregnancy in human which that conforming the first week in pregnant the cells are metamorphose and that is follow a special programmer in its growth (O'day, 2004). the most critical time for any organ is during its growth and formation of various structures (Gillbret ,2000) , this period will be very sensitive to the changer factors thus it was named the critical period for organs and tissues (O`Day, 2004) the changes affected by growth stage and concentration of the material deformation (Pastuszak,2001. The results of the present study showed the occurrence of many and the changes and phenotypic malformations in the embryos which treated with different doses of dexamethasone,Such as small size (E11\0.001mg\25gm Dex)(fig1b) and a decrease in the weight of the embryos and the body of the fetus is convoluted to be like a ball or mass of meat (E11 \0.004 mg \25gm Dex) (fig2a), the reason of low weight (table:1) comes from the great damage that occur in placenta this what we observed at (E11\0.002mg\25gm Dex) (fig 2b), this is what led to the diminishing the exchange of nutrients materials between the mother and the fetus, thus reduce protein building process and this led to the birth an embryos with low weights and decrease in the length(table 2) compared with the control group. decrease the weight of embryos, this is consistent with what's confirmed by (Siddiqui, Qamar, & Naqvi, 2013) during a study on pregnant female rats that has been treated with (4 mg / kg Dex).

In the current study, we administered a high dose of Dex large enough to cause fetal abnormalities or prenatal death. the exposure to Dex increased placental efficiency though reduced both fetal and placental weights,in pregnant mice to examine the effect of Dex exposure to severe stress during an early stages of pregnancy on the fetus development and placenta this agree with(Lee, Park, Kim, & Kim, 2012).

Malformation includes various regions for the fetus's body such as the head region like hemorrhage at E13 (all doses of Dex) (fig3b), Tumescence at the top of the head (fig3c) (E13\0.002 mg\25gm Dex)E15(0.001mg\25gm Dex)(fig3d).Short noise (fig6b),neck oedema (fig6b) , fold of skin , Wrinkled skin (E17 0.002mg\25gm Dex & P10.001mg\25gm Dex) (fig8a) the curvature of the head to another side(fig8b) and the curvature of the head towards the chest due to the curvature of the neck especially E15 (0.004 mg\25 gm Dex) to form spherical shape embryos (fig4b). Protrusion of the

eyes to forward P1(0.004mg\25gmDex)(fig6a),absent eyelids P1(0.002 mg\25gm Dex) (fig6b).

The malformations in the head region explained with the defect in the nervous system, this agree with (Lenoi *et al.*, 2013) when confirmed that the treatment with dexamethasone during the pregnancy period affected on the formation of synapses in the central nervous system and this what elucidated by (willmunt *et al.*, 1990) that any defect occur in any part in nervous system led to defect at another parts. the reason of brain hypertrophy is the pore of neural plate isn't closed, because of the neural pore is closed in E9.5 - E10 (Rice & Barone, 2000) the mice take (Dex) before the period of neural plate closing that led to prevent closing it because of the dexamethasone affected on embryos development, Most of nervous system defect comes from abnormal closing to the neural folds and called neural tube defect cases (NTD) (Al- hmood , Yosif ,2005) .take dexamethasone during pregnancy especially at organogenesis cause defect in neural folds closing irregularly and led to Neural Tube Defects (NTD) (Tayfur, 2013) this agree with what we observed in current study exacting at E17(0.002&0.004 mg\25gm Dex)(fig5b&c) .

In our results we observed The occurrence curvature in the trunk at E11(0.004mg\25gm Dex)(fig4c),E15 (0.004mg \25gm Dex)(fig4d) and with a deviation in the back bone with a swelling this is consistent with observed by (Hamoudi,2005) he notice the deformation in the trunk and deflection in the dorsal region of mice embryos when treated pregnant mice with 50mg \gm of paracetamol.Abdulmajeed. (1999) explain the swelling in the dorsal region of fetus result from rare malformation in spinal cord called Meningoen cephalo cell (MCC) As a result of the occurrence of deformation of the vertebrae leading to a swelling in the spinal cord on the dorsal surface under the body covered with skin ,Thus the (NTD) and deflection and curvature in the trunk led to formation of ball shape to embryos or spherical shape Embryos E15(0.004mg \25gm Dex) (fig4b).

At abdominal region we observed hypertrophy in liver region which clearly observed in earlier stages (E 13 and E 15) (fig:3b&d) through the body wall because of increasing hepatocyte size as a result of glycogen accumulation which what was concluded by(Al-khateeb, Barraj, & Kadhim, 2014).we also find malformation in hand pave such as fused fingers , crooked hand pave at P1(0.001&0.004mg\25gmDex)(fig7b),short fingers,loose finger at P1(0.002mg \25gm Dex) (fig7c) and the retardation of limb formation this agree with (Kim, Yun, Lee, & Kim, 2015) when they suggest that the prenatal which exposure to Dex in the mice induces fetal skeletal

malformations.This may be belong to the effect of dexamethasone on limbs formation especially Forelimb development in the mouse commences at about E9.5 with the hind limb lagging behind by about half a day.

later developmental process is mainly just for growth and maturation of the component tissues converting, for instance, the miniature embryonic cartilage template into the bony skeletal elements of the adult limb(Martin, 1990). Our observe on the limbs was the short limbs(fig8c)and abnormal leg shape such as seal leg appearance (fig 8d), We treated the pregnant mice with dexamethasone at E8 thus it affected on limbs development.

At caudal region of embryos there are various malformations occur such congestion in the end of tail (P1,0.002mg\25gm Dex) (fig6b), great convoluted in the tail and leg (E17 0.002 mg\25gm Dex)(fig4d)(fig8a)this agree with (Tayfur, 2013) .Copp *et al*(1994) explained that the delay of the closed the posterior neural pore is the main reason that leads to the tail torsion(fig8b) this delays cause stress at caudal bud because of the lack of balance between the tube neural and non-neural structure,and due to an reduction in the rate of cellular reproduction to the spinal cord (Martins-Green,1988). the exposure to prenatal(Dex) results in a placental defect as well as embryonic growth this what confirmed by(Yun, Lee, & Kim, 2016).

Time \day	Treatment (Dexamethasone mg\25gm)	
	(a)Groups (b)Group	Mean Difference (a-b)
E11	Control(0.00) 0.001	0.01 ± 0.01112
	0.002	0.02* ± 0.01112
	0.004	0.08* ± 0.01112
E13	Control (0.00) 0.001	0.14* ± 0.03333
	0.002	0.17* ± 0.03333
	0.004	0.2* ± 0.03333
E15	Control (0.00) 0.001	0.13* ± 0.09428
	0.002	0.23* ± 0.09428
	0.004	0.1 ± 0.09428
E17	Control (0.00) 0.001	0.2* ± 0.15986
	0.002	0.17*± 0.15986
	0.004	0.30*± 0.15986
P1	Control (0.00) 0.001	0.00 ±0.05774
	0.002	0.1* ±0.0577
	0.004	0.06* ±0.05774

L.S.D 0.04 .

*. The mean difference is significant at the(P =0.05), Mean± Std. Error

Table 1 :show the effect of dexamethasone on mice embryos weight at different therapeutic doses and Embryonic days. E: Embryonic day , P:Postnatal day

Time \day	Treatment (Dexamethasone mg\25gm)		
	(a)Groups	(b)Groups	Mean Difference (a-b)
E11	Control (0.00)	0.001	0.1* ± 0.07071
		0.002	0.2* ± 0.07071
		0.004	0.3* ± 0.07071
E13	Control (0.00)	0.001	0.1* ± 0.0333
		0.002	0.14* ± 0.0333
		0.004	0.14* ±0.0333
E15	Control (0.00)	0.001	0.1* ± 0.04714
		0.002	0.2* ± 0.04714
		0.004	0.00 ± 0.04714
E17	Control (0.00)	0.001	1.27* ± 0.09718
		0.002	0.14* ± 0.09718
		0.004	0.2* ± 0.09718
P1	Control (0.00)	0.001	0.2* ± 0.19149
		0.002	0.56*± 0.19149
		0.004	0.53*± 0.19149

L.S.D 0.04 .

*. The mean difference is significant at the(P =0 .05) ,Mean± Std. Error.

Table 2 :show the effect of dexamethasone on mice embryos lenght at different therapeutic doses and Embryonic days E:Embryonic day, P:Postnatal day

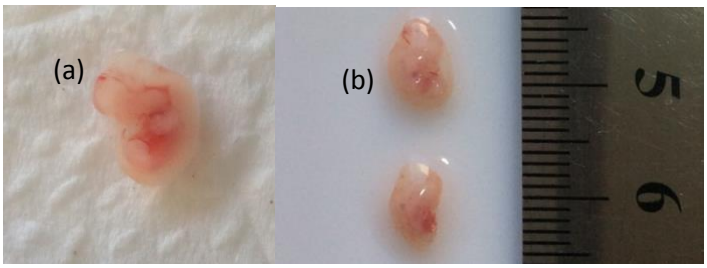

Figure1: Embryos at embryonic day 11(a)control group,(b)Treatment with 0.001mg \25gm Dexamethasone. Not the small size and different external morphology.

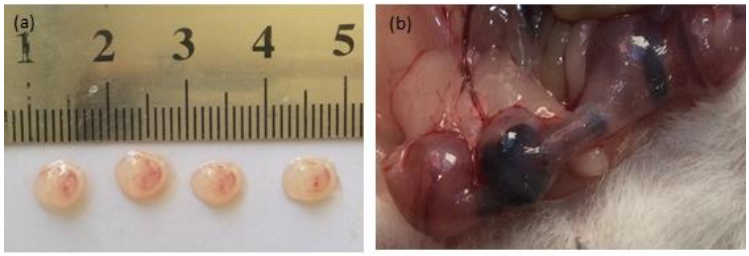

Figure 2: (a) Embryos at embryonic day 11(0.004mg\25gm Dex),(b) show the uterus of pregnant mice treated with(Dex) 0.002mg\25 gm.

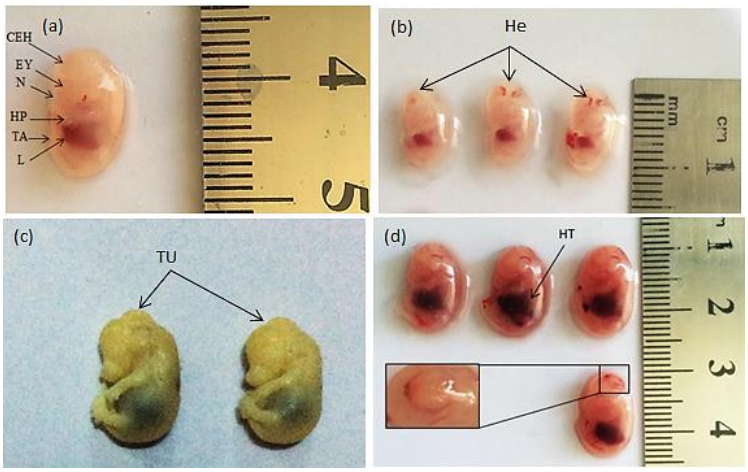

Figure3: (a)E13 control,(CEH) Cerebral hemisphere,(EY) Eye,N: Nostril, (HP) Hand plate,(TA) Tail, (L) Liver,(b) E13 their mother was treated with dexamethasone(0.001mg\25gm),(HE) Hemorrhage, (c).Embryos at E13 their mother was treated with dexamethasone (0. 002 mg \25gm).(d)(TU) Tumescence in embryo's head

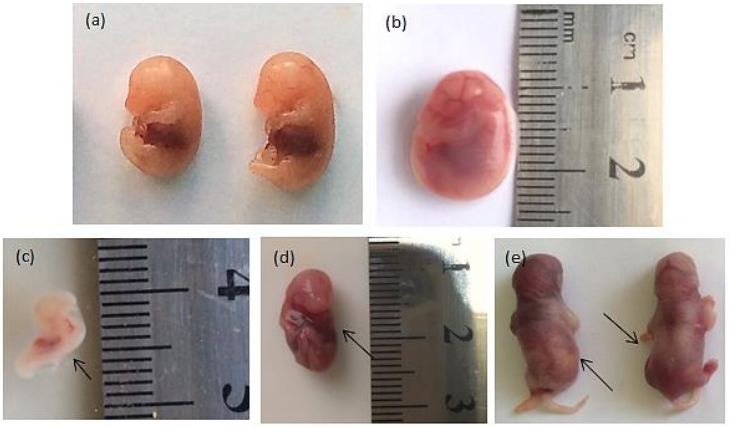

Figure4: (a) Embryos at E15 control,(b)Embryos at E15 their mother treated with (0.004mg\25gm Dex).(b)Trunk torision(arrow) at E11(0.004mg\25gm Dex).(c).E15 Trunk torision (arrow) (0.004mg \25gm),(e) P1(0.002mg\25gm Dex) Trunk torision(arrows).

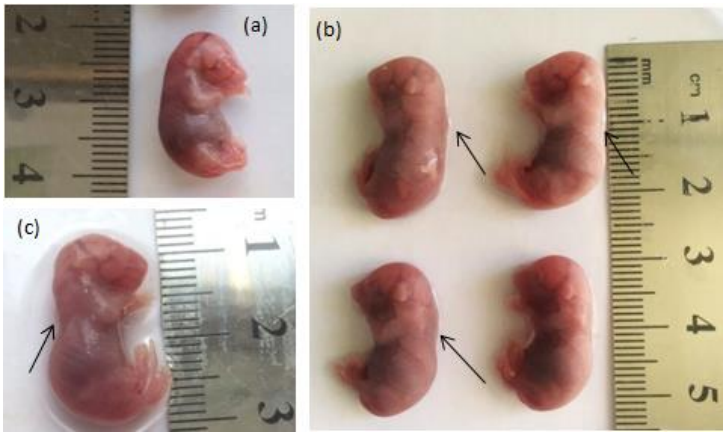

Figure5:(a) Embryos at E17 control,(b)Embryos at E17 their mother treated with (0.002mg\25gm Dex).(b),(c).E17(0.004mg\25gm) (arrows) neural tube defects .

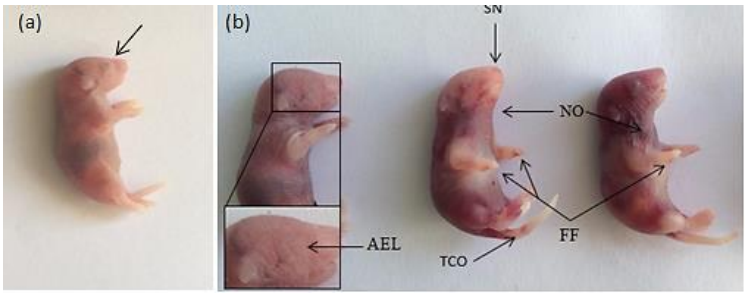

Figure6:(a) Embryos atP1 their mother treated with(0.004mg\25gm Dex) Protrusion of the eyes to forward (arrow),(b)P1(0.002mg \25 gm) (FF)Fused fingers (AF) Absent fingers (NO) Neck oedema (AEL) absent eyelids (TCO)Tail congestion (SN) Short nostril.

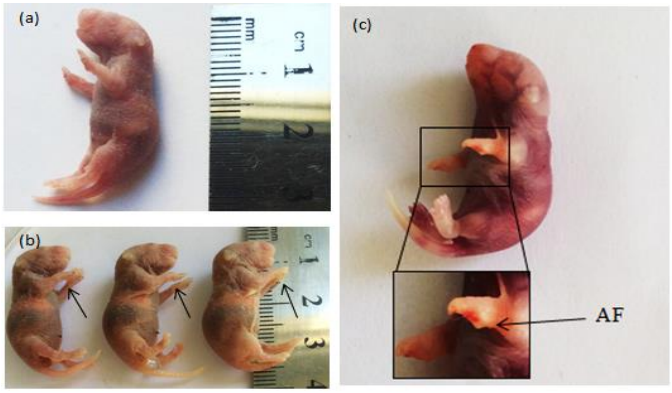

Figure7:(a)Embryos at P1 control,(b)Embryos at P1 their mother treated with(0.001mg\25gmDex)(arrows),crooked pave,(c)P1(0.002 mg\25gm Dex) (AF) absent figers.

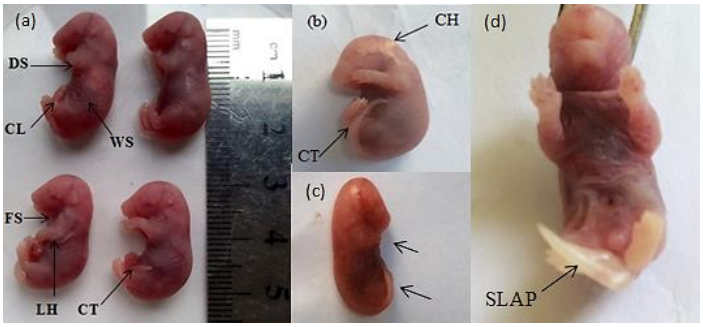

Figure8:(a)Embryos at embryonic day 17 their mother treated with (0.002mg \25gm Dex) (DS) distention in left Shoulder (CL) convoluted leg (FS) Fold of Skin, (WS) wrinkled skin (LH) long Hand (CT) convoluted tail,(b).E17 (0.002mg\25gm Dex) (CT) convoluted tail (CH)curved head to the right side .(c) short limbs (arrows).(d)P1(0.002mg\25gm Dex) (SLAP) Seal legs in appearance

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